CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

(*formerly* Scientific Agriculture)

VOLUME 34 SEPTEMBER-OCTOBER 1954 No. 5

A COMPARISON OF MOISTURE DETERMINATION METHODS FOR GRAIN CORN[1]

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[Received for publication February 3, 1954]

ABSTRACT

The moisture content of 122 samples of hybrid corn grain, ranging in moisture from 21.6 to 44.2 per cent, was determined by the Brown-Duvel, Electrical Tester and Ear Shrinkage methods. Reliability of the three methods was compared by correlation and regression analyses and the standard error of estimate. The standard error of estimate of Brown-Duvel results ranged from 0.83 to 2.52 percentage units of moisture for the E.T., and from 1.67 to 3.17 percentage units of moisture for the Ear Shrinkage method. For experimental work with corn the E.T. was considered sufficiently reliable and much more rapid than the Brown-Duvel. The Ear Shrinkage method is convenient, though less accurate than either of the other methods.

INTRODUCTION

Rapid and accurate moisture determination is essential in experimental work with corn for effectively comparing grain yields from performance trials. Methods similar to those used for estimating moisture in corn have been studied extensively with wheat. Hlynka, Martens and Anderson (2) tested moisture determination methods with 159 samples of hard red spring wheat having a moisture range of 11.5 to 17.5 per cent. Results obtained with ten electrical moisture testers were compared to those obtained with the Brown-Duvel and the two-stage vacuum oven. The standard error of estimate of 0.15 per cent for the Brown-Duvel results indicated close agreement with the vacuum oven method. The standard error of estimate of vacuum oven results for the electrical testers ranged from 0.23 to 0.50 per cent, indicating that some of the testers studied were considerably more accurate than others.

Three methods for determining moisture in corn are commonly used in Canada. These are: *first*—the Brown-Duvel oil distillation, one of the standard methods, in which the moisture in a weighed quantity of grain is vaporized by heating in oil, condensed and measured in a graduated cylinder (1); *second*—the Electrical Tester[4] method in which the moisture is indicated by the change in capacitance of a condenser when grain becomes the dielectric; and *third*—the Ear Shrinkage method in which the moisture is calculated from the weight of the ear after a period of drying. The com-

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[4] Designated throughout this article as "E.T". The Steinlite Electrical Moisture Tester, Serial No. 7748, Type S, was used in these investigations.

parative reliability of these methods has been under investigation by the Forage Crops Division since 1949. A summary of these investigations is given in this report.

MATERIALS AND METHODS

During the years 1949 to 1952 inclusive, moisture determinations were made by the Brown-Duvel, E. T. and Ear Shrinkage methods on grain samples of a number of corn hybrids. The hybrids were chosen at random from the various grain production tests and represented a wide range in moisture content. In 1949, as a preliminary investigation, the moisture of 10 hybrids was determined by each of the three methods. The following year, 34 hybrids were included in the study; in 1951 the number of hybrids was increased to 48, and in 1952 moisture tests were made on 30 hybrids. The Brown-Duvel method, being one of the official methods of determining the amount of moisture in grain, was used as the standard for comparing the reliability of the other methods. Comparisons were made by means of the *t* test and also by correlation and regression analyses.

A 10-ear sample of each hybrid was collected in the field from the border plants of the four replications of the test rows. Kernels were shelled by hand from the central portion of the ears and a well-mixed sample of approximately 400 grams, was tightly sealed in a moisture-proof jar for test purposes.

Moisture determinations were made on these samples by the E.T. method according to the manufacturer's directions within 24 hours of collection. The 100-gram sample of corn used for the E.T. was immediately tested for moisture in the Brown-Duvel tester according to the method outlined by Coleman and Boerner (1), i.e. 100 grams of corn in 150 cc. of light oil (S.A.E. 10) was heated slowly in a copper flask, to reach a temperature of 195° C. in 20 minutes, while the evaporated moisture was condensed and collected in a graduated cylinder. All determinations were made on duplicate samples and the average is reported as the moisture content for the respective methods.

The moisture content by the Ear Shrinkage method was calculated on the basis of the difference between the harvested weights of the ear samples and the weights after drying in a forced hot air dryer. Each hybrid was grown in four replicated plots of 15 3-plant hills. Between 35 and 40 ears, having a total green weight of approximately 20 pounds, were harvested from each plot. Each lot of ears was placed in an open mesh jute bag, stacked in a bin type corn dryer and dried five days at 110° F. The samples were weighed, shelled and the residual moisture (approximately 10 per cent) remaining in the grain after drying was determined by the E.T. method. This residual moisture was added to the moisture calculated from the loss in weight of the corn samples to obtain the total moisture of the hybrids as harvested. The figures reported are the averages for the four replications.

In 1952 an additional investigation was conducted to indicate the uniformity of determinations made by the Brown-Duvel and E.T. methods. Four bulk lots of corn grain, having moisture contents of approximately 10, 20, 30 and 40 per cent, were used. Six similar 100-gram samples were drawn from each lot. Moisture determinations were made on each sample with the E.T. and the same samples were immediately tested for moisture in the Brown-Duvel tester. In addition, two 200-gram samples were drawn from each of the bulk lots, dehydrated for 96 hours at 100° C., and the moisture determined by the loss in weight.

The dehydrator used for the dehydration of the bulk samples of corn was of the type commonly employed on experiment stations for the drying of forage samples of grasses and clovers to moisture-free weight. The corn was placed in a tray made of fine mesh wire which permitted heated air at 225° F. under forced draft to circulate through it freely. The temperature was controlled within a variation of ± 5° F. The moisture-free condition was determined by weighing to constant weight. Dehydrated samples tested by the Brown-Duvel method showed no further loss of moisture.

TABLE 1—COMPARISON OF MOISTURE CONTENT OF CORN AS DETERMINED BY DIFFERENT METHODS, 1949

Ear sample	Average % moisture by			Difference in moisture % from Brown-Duvel	
	Brown-Duvel	E.T.	Ear shrinkage	E.T.	Ear shrinkage
1	32.3	32.9	41.8	+ 0.6	+ 9.5
2	29.2	27.9	42.8	− 1.3	+ 13.6
3	32.6	30.8	44.1	− 1.8	+ 11.5
4	31.6	31.6	43.0	0.0	+ 11.4
5	30.9	31.1	40.6	+ 0.2	+ 9.7
6	35.2	34.3	46.1	− 0.9	+ 11.9
7	30.0	29.4	41.8	− 0.6	+ 11.8
8	31.8	31.8	39.5	0.0	+ 7.7
9	22.6	24.7	33.2	+ 2.1	+ 10.6
10	30.2	31.1	37.7	+ 0.9	+ 7.5
Mean (10 hybrids)	30.6	30.6	41.1	− 0.08	+ 10.52
t	—	—	—	0.36	9.18
t 5%	—	—	—	2.26	2.26

EXPERIMENTAL RESULTS

Moisture determinations of 10 hybrids, 1949

The average moisture content of the ten hybrids as determined by each of the three methods in 1949 is shown in Table I. This table also shows the differences in average moisture content between the Brown-Duvel determinations taken as the standard, and those of the E.T. and Ear Shrinkage methods.

The average difference between the Brown-Duvel and E.T. methods was .08 percentage units and 70 per cent of the determinations differed by less than 1 percentage units. The *t* test indicated no statistical difference between the two methods. Reference to Table 2 shows that determinations by the two methods were associated to the extent that the correlation coefficient obtained was + .95. The error of prediction of Brown-Duvel moistures from E.T. results was calculated to be 0.98 per cent moisture. While the average moisture content of the 10 samples was the same by either method, the regression coefficient of .99 indicated that the E.T. tended to underestimate the moisture.

Between the Brown-Duvel and the Ear Shrinkage determinations there was an average difference of more than 10 percentage units of moisture, which was a significant amount. The degree of association shown in Table 2 between these two methods, while highly significant (+ .86), indicated that there was more variability present in the Ear Shrinkage than in the E.T. determinations. This was also indicated by the higher standard error of estimate. The regression coefficient of 1.34 indicated that the Ear Shrinkage method considerably overestimated the moisture as determined by the Brown-Duvel method.

Moisture determinations of 34 hybrids, 1950

Of the 34 comparisons made between Brown-Duvel and E.T. determinations, 23 (68 per cent) differed by less than 1 percentage units, and 8

TABLE 2—COMPARISON OF MOISTURE DETERMINATION METHODS WITH CORN HYBRIDS IN THE FOUR YEARS, 1949-52

Year	Correlation coefficient	Regression coefficient	Standard error of estimate	Conversion equation
1949				
Brown-Duvel: E.T.	+ 0.95	0.99	0.83	$y = 0.99x + 0.07$
" " : Ear Shrinkage	+ 0.86	1.34	1.77	$y = 1.34x + 0.12$
1950				
Brown-Duvel: E.T.	+ 0.75	0.98	2.52	$y = 0.98x + 0.32$
" " : Ear Shrinkage	+ 0.64	1.32	2.97	$y = 1.32x + 0.64$
1951				
Brown-Duvel: E.T.	+ 0.91	0.99	1.83	$y = 0.99x + 0.14$
" " : Ear Shrinkage	+ 0.78	1.31	3.17	$y = 1.31x + 0.37$
1952				
Brown-Duvel: E.T.	+ 0.96	1.04	1.08	$y = 1.04x - 0.03$
" " : Ear Shrinkage	+ 0.85	1.34	1.67	$y = 1.34x + 0.19$

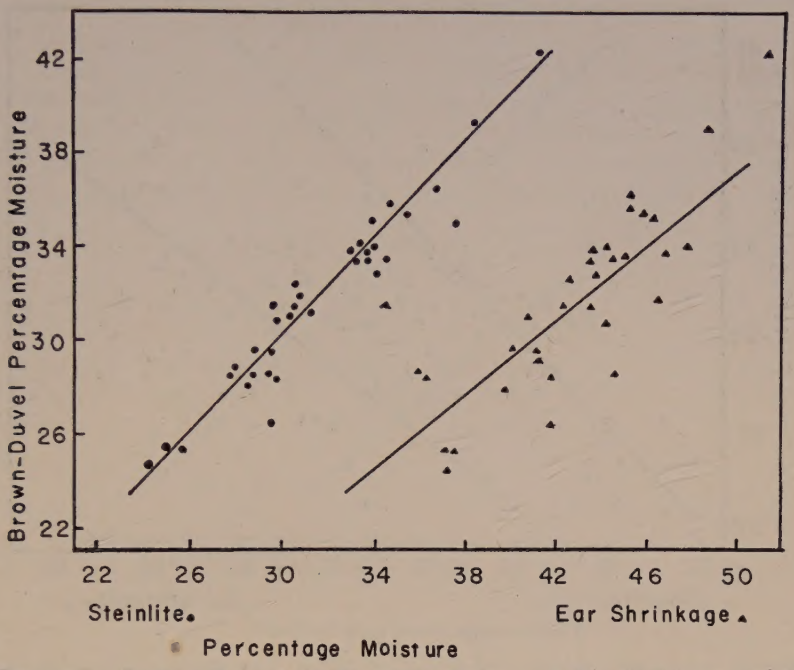

FIGURE 1. Scatter diagram of Electrical Tester and Ear Shrinkage moisture determinations in relation to Brown-Duvel results with samples of 34 corn hybrids in 1950.

(24 per cent) differed by between 1 and 2 percentage units. In comparing the Brown-Duvel data with the moisture percentages obtained by the Ear Shrinkage method the differences ranged from 3.1 to 16.0 percentage units with an average difference of 10.9 percentage units.

In Figure 1, the Brown-Duvel results for the 34 hybrids are plotted against the E.T. and Ear Shrinkage determinations in a scatter diagram along with the appropriate regression lines. In Table 2, it may be seen that the regression coefficients of .98 and 1.32 for the E.T. and Ear Shrinkage methods respectively were closely similar to those obtained in the previous year. However, the reliability of both methods was much lower since the error of estimating Brown-Duvel results from E.T. determinations was 2.52 percentage units and from Ear Shrinkage reading was 2.97 percentage units of moisture. Correlation coefficients for the association between these methods, indicate that in 1950 the reliability of the E.T. method was approximately 56 per cent compared to 41 per cent for the Ear Shrinkage method.

Moisture determinations of 48 hybrids, 1951

For this series of 48 hybrids the average difference between the Brown-Duvel moisture determinations and those of the E.T. was .02 percentage units and was not statistically significant. The average difference between the Brown-Duvel and Ear Shrinkage determinations was 10.29 percentage units with a range from 5.1 to 17.2 percentage units.

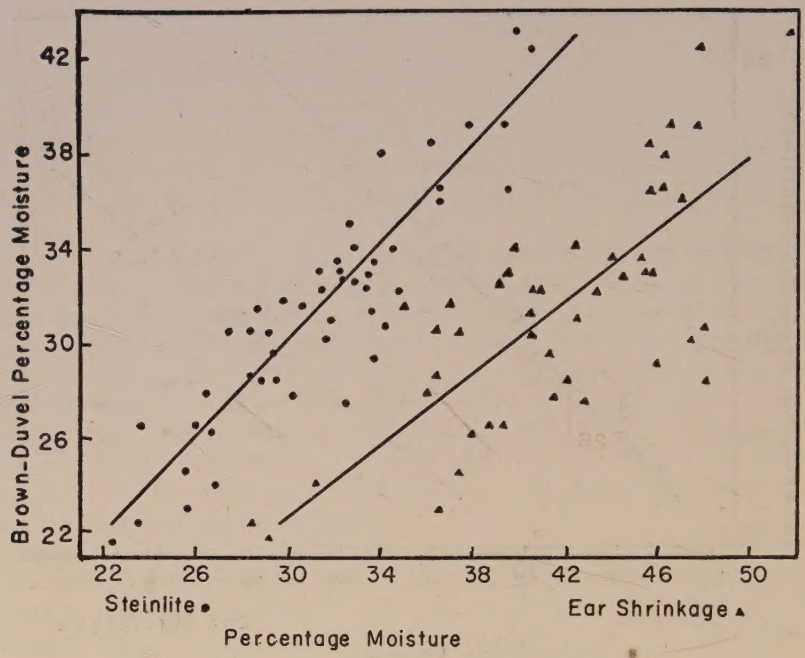

FIGURE 2. Scatter diagram of Electrical Tester and Ear Shrinkage moisture determinations in relation to Brown-Duvel results with samples of 48 corn hybrids in 1951.

Figure 2 is a scatter-diagram of the moisture determinations for the 48 hybrids by the Brown-Duvel method plotted against those for the E.T. and Ear Shrinkage methods. The respective regression lines for these methods as indicated by the regression coefficients of .99 and 1.31 (see Table 2) are also shown. The reliability of the E.T. method in comparison to the Brown-Duvel was greater than that of the Ear Shrinkage since the respective errors of prediction were 1.83 and 3.17 percentage units of moisture. The distribution of the points about the regression line is an indication of the reliability of the determinations.

Moisture determinations of 30 hybrids, 1952

The moisture determinations of 30 hybrids in 1952 showed an average difference between the Brown-Duvel and E.T. readings of 1.19 percentage units. This difference was greater than in previous years and was statistically significant by the *t* test. Individual hybrids differed by as much as 3.4 percentage units of moisture for the two methods and fewer than 30 per cent of the hybrids showed differences of less than 1 percentage units. The Brown-Duvel determinations were predominantly lower than the corresponding E.T. readings. This may have been due to the fact that in this year running water was not available to cool the condenser tubes of the Brown-Duvel tester.

The Ear Shrinkage moisture readings for the 30 hybrids averaged 10.6 percentage units higher than the Brown-Duvel determinations. Individual differences ranged from 7.6 to 13.8 percentage units of moisture.

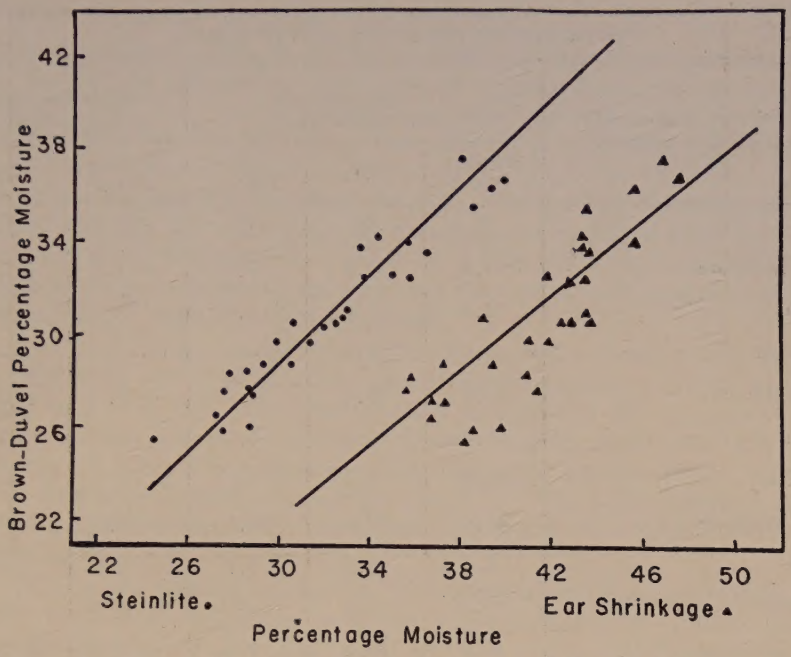

FIGURE 3. Scatter diagram of Electrical Tester and Ear Shrinkage moisture determinations in relation to Brown-Duvel results with samples of 30 corn hybrids in 1952.

The relationship between the Ear Shrinkage and Brown-Duvel readings was similar to that found in previous years, as the formula for conversion of readings showed no difference.

Figure 3 shows the Brown-Duvel readings for the 30 hybrids plotted against the E.T. and Ear Shrinkage determinations in a scatter diagram. The relationship of these methods to the Brown-Duvel is indicated by the regression lines representing a coefficient of 1.04 for the E.T. and 1.34 (see Table 2) for the Ear Shrinkage. The error of estimating Brown-Duvel results from E.T. readings was 1.08 percentage units and from Ear Shrinkage readings was 1.67 percentage units of moisture.

Moisture determinations of samples from bulk lots, 1952

Pairs of determinations made by either the Brown-Duvel or E.T. methods frequently showed some variation. Table 3 shows the variability found in sets of six determinations of moisture content on samples of corn from bulk lots at four different moisture levels. These readings are compared with the actual moisture content of the corn found by dehydration.

At every moisture level studied, the Brown-Duvel gave readings slightly lower, and the E.T. gave readings slightly higher than the actual moisture present in the corn as determined by dehydration. In most cases the range of moisture readings for the sets of six samples was rather

TABLE 3—COMPARISON OF AVERAGE MOISTURES DETERMINED BY THREE METHODS FOR SAMPLES FROM BULK LOTS OF CORN

Moisture range and variation of samples by lots	Average moisture determined by:			Deviation from dehydrator	
	Brown-Duvel	E.T.	Dehydrator	Brown-Duvel	E.T.
	%	%	%	%	%
Lot 1 Moisture	35.8	39.5	37.5	− 1.7	+ 2.0
Range	3.9	1.9	1.0	—	—
Variation	10.8	4.8	—	—	—
Lot 2 Moisture	33.2	35.6	34.7	− 1.5	+ 0.9
Range	1.0	0.9	0.5	—	—
Variation	3.0	2.5	—	—	—
Lot 3 Moisture	18.9	20.6	20.1	− 0.2	+ 0.5
Range	1.0	0.8	0.2	—	—
Variation	5.2	3.8	—	—	—
Lot 4 Moisture	8.2	10.1	9.3	− 1.1	+ 0.8
Range	0.5	0.4	0.3	—	—
Variation	6.9	3.9	—	—	—

similar for both the Brown-Duvel and E.T. methods. When this range is expressed as a percentage variation of the average reading, however, the E.T. has less variation than the Brown-Duvel at every moisture level.

DISCUSSION AND CONCLUSIONS

Yields of grain corn hybrids can only be compared reliably when the moisture content of the grain at harvest is considered. A moisture determination method to be satisfactory must be rapid, accurate and consistent. The Brown-Duvel oil distillation tester, Electrical Tester and Ear Shrinkage methods of moisture determination were compared over a four-year period, using 122 samples of grain ranging in moisture content from 21.6 to 44.2 per cent.

The Brown-Duvel is one of the official methods of testing the moisture content of corn. In accuracy, it compares very closely with the vacuum oven (2). However, it is not readily adaptable to experimental work with large numbers of samples during the harvesting period.

The E.T. provides a very rapid estimate of the moisture present in the grain. Approximately 70 per cent of the E.T. readings differed by less than 1 per cent of moisture from the corresponding Brown-Duvel results. The E.T. was considered sufficiently accurate for comparing corn yields in general experimental work.

Ear Shrinkage moisture determinations averaged more than 10 percentage units higher than the respective Brown-Duvel readings, probably due to the influence of the moisture content of the cob on the former method. However the regression coefficient between sets of readings indicated that the Ear Shrinkage method gave a farily reliable estimate of the relative moisture values of the samples. The Ear Shrinkage method was somewhat less reliable than the E.T. In the Ear Shrinkage method the moisture of the cob cannot be disassociated from the moisture of the grain and thus increases the variability. Ear Shrinkage moisture determinations may be made at any time but this would not appear to make up for the reduced accuracy of the method.

E.T. and Brown-Duvel readings both compared closely to the moisture determined by dehydration of samples at various moisture levels. Moisture estimates by the E.T. were consistently in excess of the amount actually present in the grain, while Brown-Duvel readings were generally lower than that figure. The variability in readings for samples drawn from one lot of corn was consistently less for the E.T. The E.T. therefore, would appear to be a better tester for comparing yields of a number of hybrids of similar maturity.

This study was limited to the three methods of moisture testing commonly used in experimental work with corn in Canada. Other methods of moisture determination may be of equal or greater efficiency than those compared.

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EFFECTS OF PRUNING ON SPRAY DEPOSITS FROM CONCENTRATE ORCHARD SPRAYERS[1]

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[Received for publication January 26, 1954]

ABSTRACT

In the tops of unpruned trees, DDT deposits from concentrate orchard sprayers were about half those in pruned trees. In the dormant application the concentrate machines, using 25 per cent as much oil, gave deposits in the tops of the trees equal to those of a hand-gun sprayer. In summer applications of DDT, with equal quantities of toxicant, the deposits from the concentrate sprayers were in most cases greater than those from the hand-gun sprayer. The seasonal increase in foliar density reduced the DDT deposits from the concentrate machines in the tops of both unpruned and pruned trees, but did not affect the ratio of these deposits. Pruning did not affect the oil deposits from the conventional hand-gun or the concentrate sprayers in the dormant period, or DDT deposits from the hand-gun sprayer during the summer. Apple scab control was not better in the pruned trees, presumably because the early lime-sulphur spray deposits were similar in the pruned and unpruned plots. It is concluded that, in operating a concentrate orchard sprayer, adequate pruning is required to give a good distribution of toxicant.

INTRODUCTION

Since the first concentrate sprayer was put into operation in 1946, this type of orchard sprayer has been rapidly adopted in British Columbia. In 1952, about 90 per cent of the fruit-growing acreage was sprayed by these machines. Although concentrate spraying has been generally adopted and has given good results, there remain many factors—constructional, operational and horticultural—that require further investigation in order to achieve a more uniform spray coverage.

Among horticultural practices, tree pruning probably affects the distribution and deposits of concentrate sprays more than any other. Hammer (3), Marshall (6), and Marshall and McArthur (7) have indicated the importance of tree pruning. Mitchell (8) and Davidson (2) described the work undertaken at Michigan State College on adapting mature trees to small concentrate spray machines by heavy pruning. Klingbeil (4), reporting on these investigations, stated that adequate pruning allows better penetration and more uniform distribution of spray throughout the tree, but that excessively heavy pruning is unnecessary to accomplish satisfactory coverage with concentrate applications. Subsequently, Klingbeil and Mitchell (5) reported good coverage in both tops and bottoms of large, well-pruned apple trees sprayed with lead arsenate by concentrate machines.

MATERIALS AND METHODS

Test Orchards

The orchard used for Experiment 1 contained 29 healthy Newtown apple trees, ranging in height from 20 to 25 feet, in a 30-foot diagonal

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TABLE 1.—SPRAYERS, MATERIALS, AND CONCENTRATIONS OF SPRAYS IN EXPERIMENT 1

Spray	Date	Plot	Sprayer	Materials and amounts per 100 gal. spray	
Dormant	March 30	1	Hand-gun	Dormant oil, 200-220 SSU at 100° F. Shell Helix 29	2 gal.
				Lime-sulphur, sp. gr. 1.28	4 gal.
				Soya flour emulsifier	½ lb.
		2	Turbo-mist 1950 model	Dormant oil, 200-220 SSU at 100° F. Shell Helix 29	5 gal.
				Lime-sulphur, sp-gr. 1.28	9 gal.
				Soya flour emulsifier	1¼ lb.
First summer	May 25	1	Hand-gun	DDT, 50% wettable powder Penco WB-50	1½ lb.
		2	Turbo-mist 1950 model	DDT, 50% wettable powder Penco WB-50	20 lb.
Second summer	June 11	1	Hand-gun	DDT, 97% wettable powder Pentech W-97	¾ lb.
		2	Turbo-mist 1950 model	DDT, 97% wettable powder Pentech W-97	15 lb.
Third summer	July 31	1	Bes-spray	DDT, 50% wettable powder Penco WB-50	16 lb.
		2	Turbo-mist 1950 model	DDT, 50% wettable powder Penco WB-50	16 lb.
Fourth summer	August 2	1	Turbo-mist 1950 model	Parathion, 15% wettable powder Genithion	12 lb.
		2	Bes-spray	Parathion, 15% wettable powder Genithion	12 lb.

TABLE 2.—AVERAGE DEPOSITS OF TOXICANTS FROM THE TURBO-MIST (1950) AND BES-SPRAY CONCENTRATE SPRAYERS AND THE CONVENTIONAL HAND-GUN SPRAYER ON PRUNED AND UNPRUNED TREES, EXPERIMENT 1

Spray	Date	Toxicant	Sprayer	Amount of toxicant per tree	Average deposit, microgm./cm.²					Ratio of top deposits, unpruned : pruned
					Pruned		Unpruned		Difference necessary for significance at 5 per cent level	
					Top	Bottom	Top	Bottom		
Dormant	March 30	Dormant oil	Turbo-mist	0.08 gal.	140	150	160	180	N.s.d.	1.14
			Hand-gun	0.34 gal.	170	290	150	260	55	0.88
First summer	May 25	DDT	Turbo-mist	0.16 lb.	8.3	8.1	4.5	9.6	2.26	0.54
			Hand-gun	0.22 lb.	7.7	6.2	5.4	6.7	1.64	0.70
Second summer	June 11	DDT	Turbo-mist	0.24 lb.	11.9	10.5	8.2	9.6	2.48	0.69
			Hand-gun	0.22 lb.	7.5	6.7	8.5	8.7	N.s.d.	1.13
Third summer	July 31	DDT	Turbo-mist	0.14 lb.	4.9	7.3	2.9	7.1	1.89	0.59
			Bes-spray	0.14 lb.	3.0	12.1	0.97	7.5	2.09	0.32
Fourth summer	August 2	Parathion	Turbo-mist	0.032 lb.	0.50	1.7	0.26	1.1	0.34	0.52
			Bes-spray	0.034 lb.	0.52	2.2	0.25	1.9	0.27	0.48

planting. One row of 10 trees was pruned to average British Columbia standards; and the remainder were not pruned in the year of the test and had been only lightly pruned in the years previous to the test (Figure 1) The block of trees was divided into two plots, each containing pruned and unpruned trees.

The orchard used for Experiment 2 contained 30 McIntosh apple trees, 35 years old, and varying in height from 20 to 22 feet, in a 25-foot square planting. Fifteen trees, pruned to average standards, constituted one plot. The remaining, unpruned trees, which had rather light foliage and had not been pruned for several years, made up the second.

Equipment

A conventional hand-gun power sprayer and three concentrate sprayers were used. Machine specifications were:

(*a*) A 2-gun power sprayer*, pump pressure 600 p.s.i., nozzle apertures 7/64 in.

(*b*) Turbo-mist** hydraulic concentrate machine, 1950 model. Pump pressure 300 p.s.i., output 2.5 g.p.m. Seven-nozzle manifold, disk orifices thus: top 0.046 in., second and third 0.060 in., lower four 0.040 in. Air velocity at fishtail air vent 115 m.p.h. Air volume 6800 c.f.m. Machine spraying to one side only.

(*c*) Turbo-mist concentrate machine, 1949 model, pump pressure 300 p.s.i., output 2.5 g.p.m. Seven-nozzle manifold with orifices all 0.046 in. Air volume 6000 c.f.m., air velocity at the vent 90-95 m.p.h. Machine spraying to one side only.

(*d*) Bes-spray† hydraulic concentrate machine, 1950 model, 13 h.p. blower. Pump pressure 250 p.s.i., output 3.0 g.p.m. Six-nozzle manifold with disk orifices thus: top 0.047 in., second and third 0.0625 in., lower three 0.047 in. Air velocity at air vent 100 m.p.h. Air volume 8300 c.f.m. Machine spraying to one side only although built to spray to both sides.

Sprays and Spraying Procedure

Details of application and spray materials used in Experiment 1 are given in Table 1. Calm conditions prevailed during the application of all sprays. The tractor speed during concentrate machine spraying was always maintained at 1 m.p.h. and the sprayers were operated so that the upper part of the air vent passed under the outermost tree branches. Hence the spray was driven obliquely upward through the trees. Both sides of each test row were sprayed before moving on to the next. In the hand-gun applications, the trees were sprayed from two sides. The procedure was as follows: The operator moved close to the tree trunk and with horizontal sweeps of the spray gun sprayed the inside of the opposite half of the tree, gradually working upward toward the top centre. The operator then moved back and sprayed the near side of the tree, using vertical sweeps of the spray gun. The performance was repeated from the opposite side. The trees were sprayed to the point of drip.

* Hardie Manufacturing Co., Portland, Oregon.
** Okanagan Turbo Sprayers Ltd., Penticton, B.C.
† Besler Engineering Corp., Emeryville, California.

In Experiment 2, lime-sulphur, sp. gr. 1.28, at 10 gal. per acre, was applied in five sprays to the two plots with the Turbo-mist 1949 model concentrate machine, commencing with a pink spray, May 9, followed by a calyx application, May 22, and three cover sprays, June 4, June 18, and July 3. The sprayer was operated at $1\frac{1}{4}$ m.p.h. for all applications.

Sampling and Analysing

In Experiment 1, twig or leaf samples were taken for deposit analyses before and after each spray was applied; 10 trees per plot were sampled, 5 pruned and 5 unpruned. In Experiment 2, sampling was carried out only before and after the first and third cover sprays, June 5 and July 3; 24 trees were sampled, 12 pruned and 12 unpruned.

For the dormant spray deposits, two samples of 25 twigs each were taken from each tree, one approximately 6 feet and the second approximately 20 feet above the ground. They were taken at random from all parts of the tree at each level. Fifty pieces each 10 cm. long were cut from the 25 twigs and analysed for oil deposit by the method of Pearce, Avens, and Chapman (9).

For the summer spray deposits, samples of 25 leaves were picked at the same heights as for the dormant samples. Two disks, each 2 cm. in diameter, were cut from each leaf and the 50 disks thus obtained were composited for analysis.

The potentially available sulphur in the lime-sulphur deposits was determined by a method developed at the Summerland laboratories, as follows: Boil the disks for 20 minutes in 30 ml. of solution containing 2 gm. of sodium sulphite, add 10 ml. of 20 per cent acetic acid to the cold filtrate, allow to stand 5 minutes, then add 10 ml. of 7 per cent formaldehyde and titrate with N/32 iodine solution.

The parathion deposits were determined by soaking the leaf disks in 50 ml. of benzene for 10 minutes with occasional swirling. The solution was decanted and analysed by the method of Averell and Norris (1).

The DDT deposits were removed from the disks in the same manner as for parathion. The benzene strip solution was shaken with 2 gm. of anhydrous sodium sulphate and an equal quantity of Johns-Manville Celite No. 503, allowed to stand 5 minutes, then filtered through a Whatman No. 4 filter paper. The DDT was determined in an aliquot of the filtrate by a modified Schechter-Haller (10) method.

RESULTS AND DISCUSSION

Table 2 records the deposits recovered in Experiment 1 from tops and bottoms of pruned and unpruned trees, the amounts of materials applied per tree, and the ratios of the deposits in the tops of unpruned trees to those in the tops of pruned trees.

Table 3 records the sulphur deposits in Experiment 2 for tree tops and bottoms, the amounts of lime-sulphur applied per tree, and the ratios of the deposits in the tops of unpruned trees to those in the tops of pruned trees. Apple scab control was similar in the two plots. In the unpruned trees the fruit was 91 per cent clean; in the pruned trees, 94. The unsprayed check trees produced only 18 per cent clean fruit.

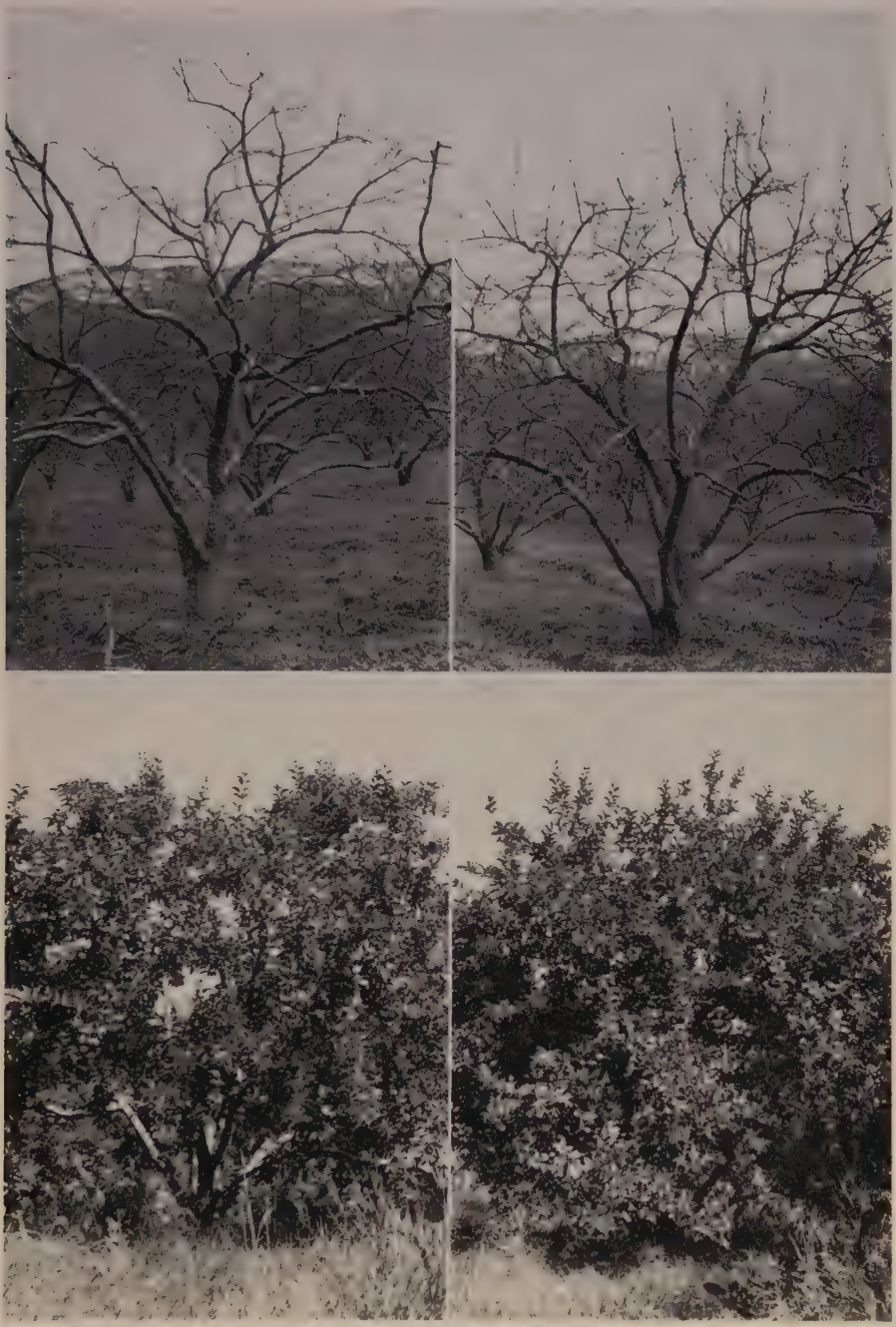

FIGURE 1. Typical trees used in Experiment 1. *Upper left*, pruned tree in dormancy; *upper right*, unpruned tree in dormancy; *lower left*, pruned tree in full foliage; *lower right*, unpruned tree in full foliage.

TABLE 3.—AVERAGE DEPOSITS OF LIME-SULPHUR FROM TURBO-MIST (1949) SPRAYER ON PRUNED AND UNPRUNED TREES, EXPERIMENT 2

Spray and date	Lime-sulphur applied per tree, gal.	Sampling level	Sulphur deposit microgm./cm.²*		Ratio of deposits, unpruned : pruned
			Unpruned	Pruned	
First cover spray June 4	0.15	Top	1.5	1.5	1.0
		Bottom	4.7	5.5	0.85
Third cover spray July 3	0.11	Top	1.1	2.0	0.55
		Bottom	4.4	4.6	0.96

* Difference necessary for significance (between sampling levels and between pruned and unpruned) at 5 per cent level: June 4 spray, 0.91; July 3, 0.55.

In the dormant spray, although only one-fourth as much oil was applied to each tree by the Turbo-mist as by the hand-gun machine, 0.08 and 0.34 gallon respectively, analysis of variance showed that the oil deposits recovered from the tree tops were not significantly different (Table 2). Evidently the concentrate method of applying dormant oil is considerably more efficient than the conventional hand-gun application. With trees in the dormant state, pruning had no significant effect on the spray deposit in either method of application.

The higher efficiency of the concentrate sprayer was evident also during the summer period but not to so great an extent. Although the amounts of DDT applied per tree by the hand-gun and the concentrate sprayers were similar, the deposits from the latter machine were, in most cases, greater (Table 2).

The effect of foliar density was noticeable even in the first summer spray, when the foliage was not very abundant. The top deposit applied by the Turbo-mist was generally less than half the bottom deposit in the unpruned trees, whereas top and bottom deposits were the same in the pruned trees. The seasonal increase in the foliar density reduced deposits in the tops of both unpruned and pruned trees. In addition, in all the summer sprays, the deposits from the two concentrate machines in the tops of unpruned trees were approximately half those from the pruned trees. However, the ratio of deposits in the tops of unpruned trees to those in the tops of pruned ones was not affected by the increase in foliar density during the season. The hand-gun application gave equal deposits in pruned and unpruned trees.

In Experiment 2 the sulphur deposits in the bottoms of pruned and unpruned trees were approximately equal in both the first and third cover sprays (Table 3), indicating that the increase in density of foliage between the two sprays had little effect on the spray deposit. Top deposits were similar for the first cover spray, but for the third cover spray the deposits were nearly twice as great in the pruned trees, presumably because of the denser foliage at the later date in the unpruned trees. Although the unpruned trees had not been pruned for several years, they were very open and the foliage was only of medium density. This probably accounts for the similar deposits in the tops of the pruned and the unpruned trees at the time of the first cover spray.

Since the top deposits in unpruned and pruned trees were similar in the first cover spray, it was not remarkable that scab control in the pruned trees was little better than that in the unpruned trees. The early sprays are as a rule the most important in apple scab control. The high deposits in the tops of pruned trees as compared to those in unpruned trees in the third cover spray had little bearing on the control of scab. Other contributing factors probably were the less dense foliage in the unpruned trees, and the fact that little late scab infection occurred during the season.

It is concluded that the horticultural practice of good pruning of fruit trees is required to obtain adequate tree-top deposits of toxicants from concentrate machines.

ACKNOWLEDGMENTS

The authors wish to thank K. Williams, F. E. Brinton, and Miss J. A. Eraut, Chemistry Laboratory, Summerland, for assistance in sampling and analyses; G. D. Halvorson, Entomology Laboratory, Summerland, and E. C. Swales, Provincial Department of Agriculture, Nelson, B.C., for operation of the equipment. They also wish to thank the district horticultural officers of the Provincial Department of Agriculture at Penticton, Summerland, Oliver, and Nelson, B.C., and various officers of the Entomology Laboratory, Summerland, for assistance, particularly in the pruning of the orchards.

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COORDINATED SOIL-PLANT ANALYSIS

II. ACCURACY AND PRECISION OF FLAME PHOTOMETRIC METHODS FOR CATION ANALYSIS[1, 2]

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[Received for publication January 29, 1954]

ABSTRACT

Semi-micro procedures were developed for flame photometric determination of exchangeable potassium, calcium and magnesium in soils, and total amounts of these elements in plant tissue. Analyses were made using a Beckman Model DU Spectrophotometer with the miniature atomizer-burner flame accessory and the photomultiplier attachment.

Results obtained with 16 standard soil samples distributed by the National Soil Survey Committee of Canada agreed closely with those obtained by collaborating laboratories for potassium and calcium, but agreement was less satisfactory for magnesium. Statistical analysis of the results of 20 duplicate photometric determinations of exchangeable cations gave co-efficients of variability of 5.5, 1.9 and 3.2, respectively, for potassium, calcium and magnesium.

The presence of phosphate seriously depressed cation emission with solutions prepared from wet ashing of plant tissue. Separating the cations by exchange resin, the average differences between photometric values and those obtained by chemical methods were + 1.0 per cent and − 5.0 per cent for calcium and magnesium respectively. Coefficients of variability in triplicate analyses of five samples ranged from 3 to 6.

INTRODUCTION

This is the second report on a project, the objectives of which have been stated previously (4). The purpose of the present investigation is to assess the accuracy of flame photometric methods of determining potassium, calcium and magnesium in soil and plant tissue, by comparing results obtained by these methods with those from standard chemical procedures, and to evaluate their precision by deriving co-efficients of variability from replicate analyses.

METHODS AND APPARATUS

A Beckman Model D U spectrophotometer with the new flame accessory (Model 9200) and the all-metal burner, was the instrument used. The photomultiplier attachment, which increases the sensitivity in the spectral region below 600 mμ, was available for part of the work. Instrumental settings and concentrations of standard solutions were similar to those reported earlier (4), with the exception that the photomultiplier permitted the use of narrower slits for calcium and magnesium (0.01 and 0.05 mm., respectively). Stock standard solutions, one each for soil and plant analyses, containing the chlorides of potassium, calcium and magnesium in 0.2 N HCl were prepared, and dilutions of these used in making up the standard curves. These latter solutions were stored in polyethylene bottles, and the standard curves were checked whenever a series of determinations was made, the curves being redrawn as necessary.

[1] Contribution from the Department of Chemistry, Macdonald College (McGill University), Macdonald College, Que. Presented before Soils Section, Agricultural Institute of Canada, Saskatoon, Sask., June 25, 1953.

[2] Macdonald College Journal Series No. 356, Macdonald College, Que.

[3] Holder of American Potash Institute Fellowship, and Professor of Chemistry, respectively.

Exchangeable cations were displaced from 5-gm. samples of air-dry soil by gravity leaching with 15-ml. portions of NH_4Ac solution through 25-ml. Gooch crucibles containing filter paper disks. This procedure simulates method A-1, employed in a collaborative study on exchangeable potassium determination by the A.O.A.C. (3). It is convenient for handling large numbers of samples.

The data for exchangeable cations by collaborating laboratories* in Table 3 were obtained by the following methods:

Cations were extracted from 30 to 50 gm. of soil with NH_4Ac in the usual way (6); calcium was determined volumetrically with permanganate titration of the oxalate; magnesium was precipitated as the ammonium phosphate salt, and measured colorimetrically or volumetrically, and potassium was precipitated as the cobaltinitrite, and estimated in most cases by titration with standard permanganate solution.

Three methods of ashing plant tissue were compared:

(1) Wet ashing by digestion with HNO_3 and finally with a mixture of HNO_3 and HCl until the silica was flocculated and the tissue particles could no longer be distinguished.

(2) Dry ashing at 300° C., finally for 5 hr. at 500°-550° C. as described by Piper (7) and Brown *et al.* (2).

(3) Dry ashing at about 600° C. in presence of H_2SO_4 (1), dissolving the ash in concentrated HCl.

For the chemical analysis of plant tissue by standard methods, calcium was determined by a single precipitation with ammonium oxalate, and titration with standard permanganate solution (1, p. 97), while magnesium was precipitated in the solution from the calcium determination by addition of $(NH_4)_2H\ PO_4$ under basic conditions, and was weighed as $Mg_2P_2O_7$ after ignition in the muffle furnace (1, p. 98).

Correction of the depressing effect of phosphate on spectral emission, was attempted in three ways:

(1) By addition to both standard and sample solutions, as suggested by Cooley (3), of sufficient phosphate to give a concentration of at least 1000 p.p.m. of this element.

(2) Addition of an excess of anion exchange resin to the tissue digests, in an effort to remove enough of the phosphate in an equilibrium reaction, to permit estimation of the cations in the supernatant solution. Nalcite S.A.R. and Duolite A-2 anion exchange resins, both saturated with hydroxyl ions, were used for this purpose.

(3) By passing the tissue digests through a cation exchange resin column, and after thorough washing, eluting the cations with 5N NH_4NO_3. Dowex-50 (Nalcite HCR) was the resin selected, just sufficient resin being used to ensure adsorption of the maximum amounts of cations expected. (The effluents from the sample solutions usually were tested in the photometer to be certain no cations were lost).

RESULTS AND DISCUSSION

The reproducibility of results using the photomultiplier as compared with the regular phototubes is indicated in Table 1. Duplicate portions of 20 corn-leaf samples were ashed by the nitric acid method, and the cations determined with the regular equipment. This procedure was repeated later, using duplicate portions of the same samples, estimating the cations determined with the use of the photomultiplier.

* *Unpublished results.* Base exchange data of standard soil samples distributed by National Soil Survey Committee of Canada. Compiled by Soil Survey Office, Central Experimental Farm, Ottawa. 1950.

TABLE 1.—COMPARATIVE VARIABILITY BETWEEN DUPLICATE ANALYSES OF 20 SAMPLES OF CORN LEAVES USING TWO FLAME PHOTOMETER ATTACHMENTS

Element	Standard deviation[1]		Coefficient of variability	
	Regular	With photo-multiplier	Regular	With photo-multiplier
Potassium	0.072	0.135	2.3	4.4
Calcium	0.019	0.0244	3.5	3.9
Magnesium at 285.2 mμ	0.037	0.0292	10.3	7.4
Magnesium[2] at 371 mμ	0.033	—	9.7	—

[1] Results expressed as per cent dry matter.
[2] Sesquioxides removed by precipitation with oxine.

In this experiment, the variability between duplicate analyses with the photomultiplier was somewhat greater for potassium and calcium, but considerably less for magnesium. In practice, magnesium emission at the 285.2 line was very difficult to measure with the regular equipment, as the wide slit setting required gave a background reading of about 70 as compared with a reading of 35 with the photomultiplier. Determination of magnesium at a wavelength of 371 mμ gave more favourable instrumental conditions, but the necessity of using the lengthy and tedious procedure for removal of iron appears to have improved the precision only slightly. Most of the determinations reported hereafter have been made with the photomultiplier attachment.

EXCHANGEABLE CATIONS

Experimental

Attempts to estimate cations directly in the NH_4Ac leachate were not satisfactory, except for potassium, and clogging of the atomizer through incomplete oxidation of the NH_4Ac was troublesome.

The destruction of ammonium acetate and dissolved organic matter by oxidation with nitric acid gave a solution that atomized more satisfactorily. Complete conversion of the salts to the chlorides by a final evaporation with conc. HCl (on the steam bath) was necessary, as the omission of this step caused a 30 per cent depression in the recovery of added calcium. Some depression in magnesium emission also occurred.

Removal, or correction for the presence of phosphate in the HCl solutions prepared from the NH_4Ac leachates (see procedure) would appear to be unnecessary, as its presence could not be detected in solutions from a number of soils, using the method of Shelton and Harper (8).

The results of Table 2, obtained by photometric estimations with the regular photocells, indicate that leaching with four 15 ml. portions of NH_4Ac solution is sufficient for non-calcareous soils and, as would be expected, that the method is unsuitable for calcareous soils.

TABLE 2.—EXCHANGEABLE CATIONS RELEASED BY LEACHING 5-GM. SOIL WITH FOUR AND EIGHT 15-ML. PORTIONS OF NORMAL NH_4Ac SOLUTION (IN M.E. PER 100 GM.)

	Potassium		Calcium		Magnesium	
	Four	Eight	Four	Eight	Four	Eight
Average of 5 non-calcareous samples	0.215	0.221	6.15	6.22	1.56	1.49
Average of 4 samples containing free carbonates	0.194	0.203	13.61	15.50	5.18	5.31

Procedure Adopted

Fifteen ml. of normal NH_4Ac solution (pH 7.0) were added to 5-gm. samples of 2-mm. soil, the mixture shaken periodically for 1 hour, and allowed to stand over night. The suspensions were shaken and filtered by gravity through No. 30 Whatman filter paper disks covered with 3-4 gm. of acid-washed sand, in 25-ml. Gooch crucibles, then leached with 4 additional 15-ml. portions of NH_4Ac solution. The leachates were reduced to small volumes, taken to dryness after addition of HNO_3 and HCL, then, after addition of 5 ml. of conc. HCl solution, taken to dryness a second time and dehydrated on the steam bath for 2 hours at 75°-80° C. The residues were taken up in 25 ml. of 0.2 N HCl solution and heated for about 2 minutes on the hot-plate to ensure complete solution of the cations. After cooling and allowing the siliceous residues to settle, the supernatant solutions were atomized in the flame photometer.

Accuracy and Precision

To evaluate the accuracy of results obtained by the above procedure, exchangeable cations were determined in 16 standard soil samples distributed by the National Soil Survey Committee of Canada, and their analyses compared with those from the collaborating laboratories. The values for

TABLE 3.—EXCHANGEABLE CATION VALUES (IN M.E./100 GM.) FOR STANDARD SOIL SAMPLES DISTRIBUTED BY NATIONAL SOIL SURVEY COMMITTEE OF CANADA

Sample	Potassium		Calcium		Magnesium	
	Mean value[1]	Phot. value[2]	Mean value	Phot. value	Mean value	Phot. value
1	0.18	0.13	5.1	5.1	0.4	0.9
2	0.29	0.28	20.3	19.5	4.1	4.8
3	0.73	0.77	21.1	18.9	10.8	10.8
4	1.01	1.03	34.7	33.8	7.3	8.5
5	0.14	0.13	19.9	18.3	4.9	5.0

[1] Mean of values obtained by all collaborators.
[2] Obtained by flame photometry.

TABLE 4.—MEAN VALUES (M.E./100 GM.) AND VARIATION BETWEEN METHODS FOR THE EXCHANGEABLE CATION CONTENT OF 16 SOIL SAMPLES

Cation	Mean values		Standard deviation between methods within samples	Coefficient of variability
	Chemical[1]	Phot.		
Potassium	0.356	0.361	0.024	6.7
Calcium	16.14	16.14	1.35	8.3
Magnesium	3.98[2]	4.70[2]	0.72	16.6

[1] Mean values from either 4 or 5 laboratories.
[2] Significantly different at a probability of 0.01.

TABLE 5.—FLAME PHOTOMETRIC VALUES IN M.E./100 GM. FOR EXCHANGEABLE CATIONS IN DUPLICATE EXTRACTS FROM 4 SOIL SERIES (MEANS OF 5 SAMPLES)

Soil series	Potassium		Calcium		Magnesium	
Grenville	0.282	0.280	8.57	8.75	2.36	2.39
Chicot	0.235	0.239	6.32	6.45	1.51	1.56
Macdonald	0.284	0.295	5.07	5.06	1.25	1.28
Chateaguay	0.300	0.322	7.98	8.10	2.11	2.12

5 of these samples are given in Table 3, and the mean values for the entire group, together with the variation found between methods, are presented in Table 4. For potassium and calcium the means of the two methods agree closely, and the variability would appear to be satisfactorily low. There is a significant difference in the mean values for magnesium and variability is considerably greater. However, there were also large differences in the values reported for chemical determination of magnesium from different laboratories.

To obtain data on the precision of the photometric procedure, five samples from each of four soil types (differing widely in textures) were extracted in duplicate, and their cation contents determined using the photomultiplier attachment. The results are summarized in Table 5, and the coefficients of variability calculated from the analysis of variance are shown in Table 6. These results indicate that the precision of the procedure for all three cations is at least as good as that generally obtained for these determinations by standard chemical methods.

Total Cations in Plant Tissue

Experimental

Piper (7) has outlined the dangers and necessary precautions in the ashing of plant materials in the dry way and recommends wet ashing for nearly all the metallic cations. Since the former method continues to be used, and its convenience is appealing, its suitability was further examined.

TABLE 6.—VARIABILITY BETWEEN DUPLICATE DETERMINATIONS OF EXCHANGEABLE CATIONS IN 20 SAMPLES OF SOIL BY FLAME PHOTOMETRY

Cation	Mean content m.e./100 gm.	S.D.	Coefficient of variability
Potassium	0.280	0.0154	5.50
Calcium	7.04	0.136	1.93
Magnesium	1.82	0.0586	3.20

TABLE 7.—MEAN CATION CONTENT (AS PER CENT OF DRY MATTER) IN 5 CORN LEAF SAMPLES, USING DIFFERENT METHODS OF DIGESTION

Element	Wet ashing with HNO_3	Dry ashing, Piper	Dry ashing, A.O.A.C.
Potassium	3.06	2.52	2.93
Calcium	0.64	0.61	0.75
Magnesium	0.46	0.37	0.45

Flame photometric analysis of corn tissue solutions prepared by two modifications of the dry ashing technique and by a wet-ashing method utilizing nitric and hydrochloric acids are reported in Table 7. In all cases the final solution was made in 0.2 N HCl. The dry ashing method of Piper evidently results in loss of potassium by volatilization, and apparently renders part of the magnesium insoluble in 0.2 N HCl. The A.O.A.C. method of ashing appears to retain the potassium, and treatment of the ash with concentrated acid seemingly dissolves all the magnesium, but calcium results are high. It will be shown later that the presence of phosphate depresses calcium emission, so that all these results for this element are quite probably too low. The relatively high values for the A.O.A.C. method of ashing may be due to partial loss of phosphorus upon heating in presence of sulphuric acid.

The possibility of using H_2O_2 or perchloric acid along with or following the nitric and hydrochloric acid treatment for breaking down plant materials more quickly was investigated. The use of these reagents was abandoned, it being found that the presence of even a few drops of perchloric acid enhanced calcium emission in a solution containing 100 p.p.m. of calcium by 10 per cent, and addition of 2.5 ml. of 30 per cent H_2O_2 (high purity reagent) to 10 ml. of a 75 p.p.m. calcium solution gave only 80 per cent recovery (at least partly due to presence of phosphate).

Complete destruction of all traces of organic matter may not have been obtained with the HNO_3 and HCl mixture, but three successive digestions, with additions of 10 ml. HNO_3 and 5 ml. HCl each, gave no greater recovery of cations that one such digestion. As a precautionary measure, two additions of nitric acid were made in the west digestion method adopted.

Results of a study of the influence of phosphate in decreasing the intensity of calcium emission agreed closely with those of Cooley (3). Smaller depressions also were found for magnesium and potassium lines, the latter being only slightly affected. Addition of 1000 p.p.m. of phosphorus as phosphoric acid to standard and to sample solutions prepared from nitric acid digestion did not produce satisfactory results—the calcium values being much too high.

Attempts to remove phosphate by an equilibrium reaction with excess anion exchange resins were also unsuccessful.

The values for calcium and magnesium obtained by standard chemical procedures, and by flame photometric methods (with the photomultiplier) after separation of the cations from the nitric acid digest by resin exchange, are given in Table 8. The results are considered as satisfactory.

The magnitude of the interference from phosphate (possibly other anions interfere as well), is indicated in Table 9, which gives the cation analysis obtained with and without separation by the exchange resin. These results agree with the findings of the phosphorus study mentioned above in that calcium values exhibit the greatest differences, with those for potassium being negligible. In view of this latter point, direct estimation of this element in the original digest is justified, and use of a resin column that completely separates only calcium and magnesium is sufficient.

TABLE 8.—CALCIUM AND MAGNESIUM CONTENT OF DIFFERENT TYPES OF PLANT TISSUE BY CHEMICAL AND PHOTOMETRIC METHODS OF ANALYSIS (PER CENT OF DRY MATTER)

Material	Calcium		Magnesium	
	Photometric	Volumetric permanganate	Photometric	Gravimetric as $Mg_2P_2O_7$
Clover (immature)	1.38	1.43	0.52	0.54
Nettle leaves	3.89	3.61	0.81	0.86
Mangel leaves	1.90	1.85	1.49	1.66
Corn leaves	0.69	0.73	0.42	0.47

TABLE 9.—CATION CONTENT OF PLANT MATERIALS (AS PER CENT OF DRY MATTER) DETERMINED IN SOLUTIONS BEFORE AND AFTER SEPARATION BY EXCHANGE RESIN

Material	Solution	K	Ca	Mg
Clover (immature)	Original	1.95	0.91	0.45
	Eluate	2.00	1.38	0.52
Nettle leaves	Original	2.90	2.59	0.74
	Eluate	3.03	3.89	0.81
Mangel leaves	Original	4.10	0.98	1.26
	Eluate	4.05	1.90	1.49
Corn leaves	Original	2.03	0.41	0.37
	Eluate	1.91	0.69	0.42

These results also suggest that phosphate depression is more serious with the present flame attachment than with previous models (2). Further, it has been the experience of one of the authors, in analysis of forage and other materials containing phosphate with a Perkin-Elmer Model 52-A instrument, that this interference was of the order of only a few per cent.

Procedure Adopted

0.25-gm. samples of oven-dry material (heated at 105° C. for 10-12 hr. and cooled over conc. H_2SO_4) were weighed out quickly to the nearest milligram and digested with 10 ml. of conc. HNO_3 in covered 50-ml. beakers on a hot-plate, until only a few ml. remained. After addition of another 10 ml. of HNO_3 and 5 ml. conc. HCl, the mixtures were again reduced to small volumes, the covers and sides of the beakers washed down with distilled water, and the solutions taken to dryness on the hot-plate. The residual salts were converted to chlorides, and oxides of nitrogen were expelled by addition of 5-10 ml. HCl, and evaporation to dryness on the steam bath. The cations were dissolved in 25 ml. of 0.2 N HCl by heating briefly on the hot-plate, and after cooling, 10 ml. aliquots were passed slowly through columns (4 mm. in diameter by 80 mm. in length) of Dowex-50 cation exchange resin (saturated with NH_4^+). After washing thoroughly with distilled water, the cations were eluted with about 50 ml. of 5 N NH_4NO_3 solution, at the rate of 1 ml. per minute. (Completeness of cation elution was tested occasionally by comparing photometric readings obtained from the last few ml. of eluates with those from the eluting solution.) Excess ammonium salts were destroyed by digestion with HNO_3 and HCl, the solutions evaporated to dryness, and the salts converted to chlorides as before. Following dissolution in 10 ml. of 0.2 N HCl, calcium and magnesium were determined photometrically. The estimation of potassium was made from the original tissue digest.

Accuracy and Precision

The accuracy of this procedure has already been indicated in Table 8. The rather consistent discrepancy between the two sets of data for magnesium may be due to the presence of manganese in the pyrophosphate precipitate.

The precision of the results obtained with the photometric procedure is compared with that of the chemical methods in Table 10. It is felt that the values obtained substantiate the reproducibility of the adopted method.

TABLE 10.—VARIABILITY OF RESULTS OF CHEMICAL ANALYSIS OF PLANT TISSUE BY DIFFERENT METHODS (5 MATERIALS ANALYSED IN TRIPLICATE)

Element	Coefficients of variability	
	Conventional method	Photometric method
Calcium	5.64	3.16
Magnesium	2.84	6.23
Potassium (original solution)	—	6.00
Potassium (resin purified)	—	4.57

The results of the cation determinations on corn-leaves reported in the first of this series of papers were estimated in solutions prepared by the wet ashing method without removal of phosphate, and are, therefore, in error. However, analyses of these samples for phosphorus do not indicate there is sufficient variation in the level of this element to cause differential errors in different samples.

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WHEAT YIELD AND USE OF MOISTURE ON SUBSTATIONS IN SOUTHERN SASKATCHEWAN[1]

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[Received for publication February 18, 1954]

ABSTRACT

Twelve years' data on the use of moisture by wheat crops at seven Experimental Substations were summarized. The linear regression of yield on evapotranspiration showed an increase of 3.5 bushels per acre for each additional inch of water used by the crop. The relationship was actually curvilinear so that for high evapotranspiration the increase per inch was approximately 6 bushels per acre. These increases resulted not only from the direct benefits of moisture availability, but also from all factors associated with evapotranspiration that were favourable to the crop.

The estimate of yield on the substations was significantly improved when rainfall and stored moisture were used as separate variables. The regression coefficient for rainfall was 1.5 times that for stored moisture. This ratio was greater than that obtained from long-time tank and field experiments at Swift Current.

The total variance in yield was divided into parts due to differences between substations, between seasons and residual variance. After crop yields were adjusted for differences in moisture use on the basis of the residual variance, the remaining differences in yields between substations were just short of significance, and those between seasons were highly significant.

Yields of wheat on fallow were not as highly correlated with total (24-month) precipitation as with evapotranspiration because of variability in moisture storage during the 21-month summerfallow period.

The Soil Research Laboratory has studied the conservation and use of soil moisture on substations in southern Saskatchewan since 1937. The conservation in summerfallow and stubble fields at certain stations was reported recently (9). The present paper deals with the yields obtained and the efficiency of moisture use on most of the same stations. This work, conducted on farmers' fields, serves as a check on the more detailed measurements made in tanks and on plots at the Swift Current Experimental Station. In addition, it gives a better mean of the crop conditions in the area than is possible from fields at a single location.

The yield data obtained at 16 locations in southern Saskatchewan for the period 1938-46 were reported previously (8). It was shown that a wheat crop using 10.5 inches of moisture (evapotranspiration) yielded 14 bushels per acre, and that the yield increased by approximately 6.5 bushels per acre for each additional inch of water above 10.5 inches. Cole and Mathews (1), on the basis of some 96 station-years results (1907-1919) on the Great Plains of the United States, obtained a correlation coefficient of 0.76 between the yield of wheat and the total quantity of water used. The increase in yield for each extra inch of evapotranspiration was 2.5 to 5.0 bushels per acre. In later work Cole and Mathews (3) showed that the yield of spring wheat was highly correlated with the depth to which the soil was moist at seeding time.

The relationship between the yield of wheat and the precipitation received both during the current growing season and pre-seasonal, was reported by a number of authors (5) (2) (7).

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[2] Physicist and Soil Physicist, respectively.

METHODS

The soil moisture data used in this report were obtained in the detailed sampling described in the earlier paper on moisture conservation (9). The samples were taken at seeding time and harvest in wheat fields in 2-year rotations of wheat and summerfallow. Well-drained areas, 10 by 20 yards, were chosen in fallow fields on each station, and 6 sites were sampled to a depth of 4 feet on each area, viz., at the corners and the mid-points of the sides. Five samples, representing depths 0-6, 6-12, 12-24, 24-36 and 36-48 inches, were taken at each site. The apparent specific gravity of the soil was obtained so that the percentage moisture used from the soil from spring to fall could be converted to inches of water.

Crop yields were obtained from 2 square-yard samples of crop near each of the six sampling sites chosen on each station. The yields were usually in good agreement with the mean obtained by the farm-operator for the whole field. The field yields were substituted in a few cases where fields were swathed before samples could be taken.

Daily precipitation records were kept on each station and these were kindly supplied to the authors by the substation supervisors. The precipitation received between seed-time and harvest of the crop is referred to as seasonal precipitation. The total moisture used, or the evapotranspiration, is the moisture taken from the soil by the crop plus the seasonal precipitation.

The present analysis is based on the results from seven substations on medium-textured soils for a 12-year period 1938-50, omitting 1943. The soils at six of the stations were loams ranging from fine sandy loam to clay loam. The soil at the other station was classified as Sceptre clay, but the moisture characteristics of samples from some sites on the farm were little different from those of the better loams.

Thoroughness of cultivation and the amount of weed growth on the substations enter into the efficiency of moisture use in much the same way as they do in moisture conservation. Variations in crop stand due to faulty seeding or poor germination, or moisture losses due to weed growth all contribute to the errors of the measurements. The results are believed to be a good estimate of evapotranspiration and yield in the districts represented.

RESULTS

Relation Between Yield and Evapotranspiration on Substations

The mean moisture taken from the soil, the seasonal precipitation and the mean crop yield for the seven stations are given in Table 1. No samples were taken in 1943 because of shortage of help during wartime. The mean moisture used from the soil (4.11 inches) was near the 4.00 inches reported previously (9) for conservation in summerfallow. It was also about the same as the mean used from the soil by fallow wheat on plots at Swift Current for a 28-year period*. In most seasons the soil in the root zone (about 4 feet deep) was dried to the wilting point or below at harvest time, so that over a period of years the moisture stored at seed-time and the moisture used by the crop were nearly equal.

* Staple, W. J., and J. J. Lehane. Weather conditions influencing wheat yields in tanks and field plots. (*In press*).

TABLE 1.—MEAN MOISTURE AND YIELD DATA FOR 7 STATIONS, 1938-50

Year	Moisture used by crop			Yield
	From soil	Rainfall	Total	
	in.	in.	in.	bu./ac.
1938	3.79	6.26	10.05	11.4
1939	3.78	8.64	12.42	26.5
1940	4.73	6.86	11.59	21.9
1941	4.78	6.00	10.78	12.2
1942	3.88	9.96	13.84	37.8
1944	6.13	7.58	13.71	31.4
1945	5.64	3.42	9.06	11.8
1946	3.64	5.41	9.05	14.2
1947	3.81	5.89	9.70	11.0
1948	4.32	4.74	9.06	11.7
1949	2.66	5.36	8.02	6.4
1950	2.22	7.53	9.75	18.3
Mean	4.11	6.47	10.58	17.9

A scatter diagram in which yields in bushels per acre are plotted against the total evapotranspiration is shown in Figure 1. The correlation between yield Y and evapotranspiration M was 0.830 and the linear regression equation was

$$Y = 3.52\ M - 19.3 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1).$$

The regression coefficient and the intercept on the M axis were in fair agreement with the 28-year field results obtained at the Swift Current Experimental Station.

The results in Figure 1 as well as those published for all substations sampled prior to 1947 (8), suggest a curvilinear relationship between yield and moisture use. In the earlier analyses, the mean yield corresponding to an evapotranspiration of 10.5 inches of water was 14 bushels per acre, and for each additional inch the yield increased by 6 to 7 bushels per acre. For moisture use less than 10.5 inches, the yield per inch of water was somewhat lower, the curved regression line cutting the M axis at approximately 4 inches. These data are still as useful and convenient as any available for making rough estimates of yield on the basis of evapotranspiration. The yields predicted by the linear regression with slope 3.52 are satisfactory for small values of M but are too low in wet years. When a term M^2 was included in the regression, the multiple correlation coefficient

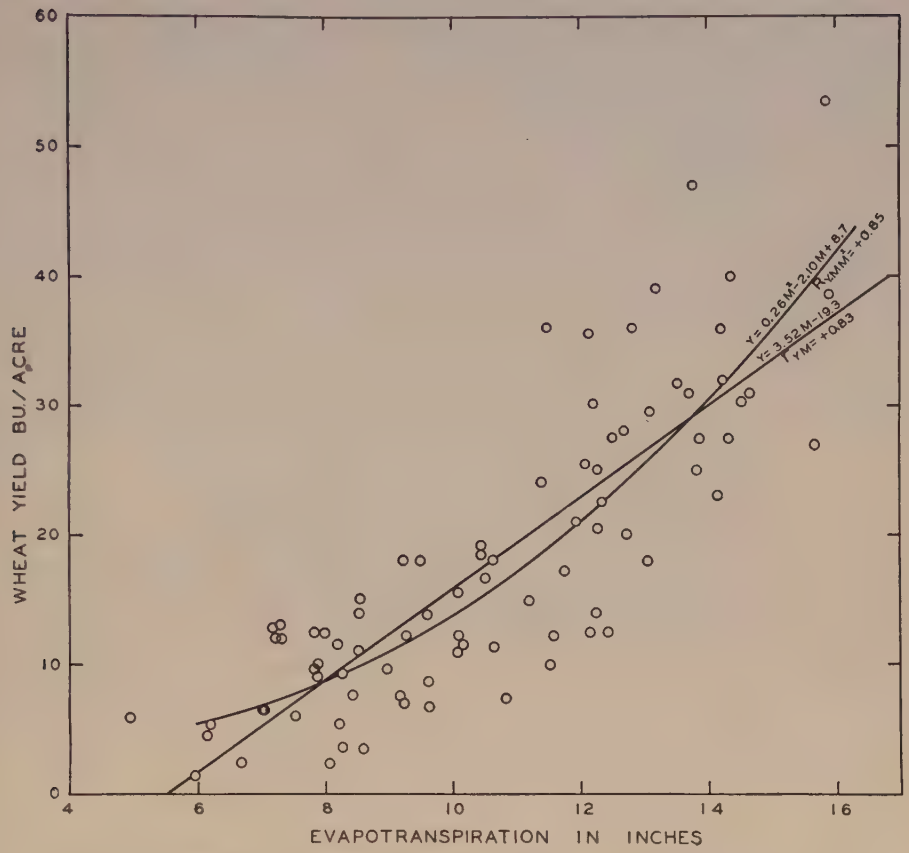

FIGURE 1. Relationship between wheat yield and evapotranspiration on substations—1938-50.

was 0.847 and the residual variance was significantly reduced. The curve corresponding to the equation

$$Y = 0.26\ M^2 - 2.10\ M + 8.7 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

is shown in Figure 1. Separation of M into seasonal precipitation P and stored moisture used S produced a comparable improvement in the estimate of yield. The resulting regression equation was

$$Y = 3.94\ P + 2.67\ S - 18.6 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

In long-time experiments at Swift Current the M^2 term likewise produced significant improvement in the regression of yield on evapotranspiration in field plots but not in tanks*. Considering M as separate variables P and S did not improve the estimate of yield in either tanks or field plots at Swift Current.

Although the equations with multiple regression give the best fit to the data available on the substations, it is possible that certain favourable conditions associated with high evapotranspiration or high rainfall, are

See footnote, page 461.

TABLE 2.—TEST OF SIGNIFICANCE OF STATION AND SEASON EFFECTS

Source of variation	Yield sum of squares	R^2	Errors of estimate			F	5 per cent point
			DF	Sum of squares	Mean squares		
Station and error	3486.43	0.5058	70	1723.09			
Error	2335.28	0.3822	64	1442.78	22.54		
Difference for testing station effects			6	280.31	46.72	2.07	2.24
Season and error	9411.14	0.6978	75	2844.25			
Error	2335.28	0.3822	64	1442.78	22.54		
Difference for testing seasonal effects			11	1401.47	127.41	5.65	1.94

producing spurious effects when only 12 years' results are considered. For instance, in Figure 1 the upper part of the figure may be unduly influenced by the high efficiencies in 1942, and to some extent in 1944, when the evapotranspiration was high. The effects of P, S or M as used here include the contributions of other seasonal weather factors associated with precipitation or stored moisture. The present authors have suggested, also, that the regression of yield on seasonal rainfall and stored moisture use may be influenced by soil texture (6). The exact nature and magnitude of these relationships will be known only when more results are available. In the meantime rough estimates of yield can be made using the method published previously or by applying the regression equations given above.

Influence of Locality and Season on Yield

In addition to sampling errors, part of the scatter of experimental points in Figure 1 may be attributed to differences between:

1. Substations (soil texture, weather, etc.)
2. Seasons (magnitude and distribution of weather factors)
3. Cultural practices (uniformity of seeding, weed control, etc.).

Differences between substations would be expected to contribute somewhat to the variability although the stations represent a rather narrow range of soil texture, and being within 100 miles of Swift Current have comparable weather in any one season. Seasonal effects are known to be important factors, depending on the distribution of rainfall and other weather conditions during growth of the crop. The effect of different cultural practices is hard to assess, and must be associated with the variations between substations. All the substation operators are good farmers but differences in yield are certain to occur due to different cultural methods.

It is convenient to separate the total variance in yield into differences between stations, between seasons and residual or error variance. Part of each variance may be accounted for by differences in rainfall and stored moisture used, and analysis of covariance enables one to test the significance of station and season effects (4) after yields are adjusted for these differences. It may be seen from Table 2 that after adjustment for P and S the differences between stations were just short of significance at the 5 per cent level (F 2.07 compared with 2.24 required) and those between seasons were highly significant (F 5.65 compared with 2.54 required at 1 per cent level).

The actual yields of wheat and those estimated in the above analysis on the basis of the error variance are shown in Table 3. The estimates were somewhat different from those given by the regression equation in the preceding section based on the total variance. The results for the different stations at the bottom of the table suggest that the mean error of estimate (actual-estimated yield) may vary with soil texture. The mean error ranges from $-$ 2.0 bushels per acre for the sandy loam at Valijean to $+$ 2.9 bushels per acre for Sceptre clay at Gravelbourg. Favourable rainfall distribution at Kincaid in a number of years undoubtedly improved the efficiency at that station. The differences in mean error of estimate for the 12 years, given in the last column of the table, are in agreement with general observations on moisture efficiency. In seasons with large negative errors the crops suffered at some stage from lack of moisture because

TABLE 3.—ACTUAL YIELDS AND ESTIMATES BASED ON ERROR VARIANCE, 1938-50

Location and soil class	Valjean Ch SL and Ht FL		Tugaske W.L.		Riverhurst Fx Si CL		Shaunavon Hr CL		Kincaid Fx Si CL and Hr CL		Swift Current Wm CL		Gravelbourg Sc C		Actual-estimated (mean)
Year	Actual yield	Estimate	Actual yield	Estimate	Actual yield	Estimate	Actual yield	Estimate	Actual yield	Estimate	Actual yield	Estimate	Actual yield	Estimate	
	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.	bu./ac.
1938	6.0	11.1	10.0	20.1	6.5	10.2	7.0	14.4	25.0	26.7	9.6	12.3	18.0	22.8	− 5.3
1939	20.0	22.2	30.7	27.4	12.3	13.2	29.5	23.8	36.0	23.8	31.8	23.6	25.0	22.5	+ 4.1
1940	13.8	15.7	20.5	21.3	11.5	12.8	35.9	25.5	27.5	25.5	18.5	17.3	25.5	21.3	+ 2.0
1941	11.4	17.9	14.0	21.1	1.5	8.1	12.5	21.5	25.1	24.6	3.5	13.1	17.2	19.7	− 5.8
1942	27.5	24.5	38.6	30.9	36.1	21.3	30.1	22.2	53.4	29.1	39.0	24.9	40.0	26.5	+ 12.2
1944	27.5	21.9	27.0	28.3	24.0	19.5	32.0	24.7	31.0	25.7	47.0	24.9	31.0	24.1	+ 7.2
1945	12.0	10.0	22.5	19.9	5.3	7.9	7.4	17.6	14.9	17.6	7.6	12.2	12.8	9.4	− 1.7
1946	12.2	20.2	18.0	15.4	12.4	12.4	15.0	12.9	13.9	12.9	15.6	16.4	12.0	11.4	− 0.4
1947	12.4	21.5	12.2	16.8	5.4	13.3	6.7	15.7	13.0	10.7	10.9	16.2	16.7	17.6	− 4.9
1948	9.6	14.8	6.5	9.1	4.5	7.5	8.7	16.3	12.2	14.3	19.2	17.3	21.0	20.0	− 2.5
1949	9.6	11.2	3.6	13.2	2.4	10.2	9.3	14.1	2.4	12.7	7.6	14.6	10.0	12.4	− 6.3
1950	28.0	22.9	11.1	14.4	6.0	6.0	18.0	15.5	18.0	19.3	11.5	17.9	35.6	22.5	+ 1.4
Mean	15.8	17.8	17.9	19.8	10.7	11.9	17.7	18.7	22.5	20.2	18.5	17.6	22.1	19.2	
Actual-estimated	− 2.0		− 1.9		− 1.2		− 1.0		+ 2.3		+ 0.9		+ 2.9		

of low rainfall, poor rainfall distribution or weed growth. Large positive values appeared in years when the precipitation was ample and well distributed. The high yields in 1942 appeared at the top of Figure 1, and contributed to the curvilinear relationship as well as to the high efficiency of seasonal rainfall. That high efficiency is related to high evapotranspiration is shown by a comparison of the evapotranspiration in column 4, Table 1 with the mean errors of estimate in the last column of Table 3.

Relation Between Yield and Precipitation for Summerfallow Period

The mean moisture conservation for the 21-month summerfallow period on the substations for the years 1938-50 was about 4 inches or 21 per cent of a mean precipitation of nearly 19 inches. This stored moisture coupled with the seasonal precipitation was highly correlated with the resulting crop yield. It is of interest to examine the relationship between yield and total precipitation for the summerfallow cycle considering preseasonal and seasonal precipitation taken together and separately. The correlation coefficient between yield and total 24-month precipitation P_{24} was 0.606 and the regression equation was

$$Y = 1.50\ P_{24} - 20.3 \quad \ldots\ldots\ldots\ldots\ldots\ldots (4).$$

When the precipitation was divided as suggested above, the multiple correlation coefficient was 0.788 and the regression equation was

$$Y = 3.71\ P + 0.70\ P_{21} - 19.4 \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

where P was the precipitation in the 3-month growing season, and P_{21} was the precipitation in the 21-month conservation period. The regression coefficient for seasonal rainfall 3.71 was close to the value of 3.94 found in Equation 3 for the coefficient of P when stored moisture S was used as the other independent variable. The coefficient 0.70 of P_{21} was 26 per cent of the coefficient 2.67 of S. Lacking a measurement of stored moisture at seeding time, estimates of yield can be made using P_{21} in the above equation, or by considering S in Equation 3 to be approximately one-fourth of P_{21}.

Actually the relation between S and P_{21} is not a constant ratio. The conservation in summerfallow depends to a large extent on the amount and distribution of precipitation. When stored moisture is plotted against the 21-month precipitation the points are widely scattered—a straight line fitted by least squares has a slope of 0.2 and cuts the P_{21} axis near the origin. Moisture from continuous or intermittent rain of 0.4 inch or more is stored efficiently, but light, widely spaced showers are often ineffective. Moisture conservation is a dynamic process and for accurate estimates the sequence of weather changes throughout the year must be considered. Eventually, for a given soil and cultural treatment, it should be possible to compute the potential yield from meteorological data collected from one harvest to the next.

ACKNOWLEDGMENTS

The authors wish to express appreciation to P. J. Janzen and N. A. Korven for supplying precipitation and other relevant data for the substations in the area served by the Swift Current Experimental Station. A. U. Earl assisted with much of the soil and crop sampling, and laboratory analyses. J. L. Doughty contributed in many ways to the carrying out of the experimental work and the preparation of this report.

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TRICHOTHECIUM FOLIAGE ROT OF GREENHOUSE CUCUMBERS[1]

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[Received for publication February 4, 1954]

ABSTRACT

Trichothecium roseum (Hoffm.) Link ex Fr. causes a foliage rot of greenhouse cucumbers in southern Ontario. The fungus develops saprophytically for a time upon decaying organic matter such as insect excreta and abscissed blossoms, prior to its parasitic invasion of adjacent healthy leaf tissue. The greatest damage occurs on tender foliage in the presence of high humidity.

INTRODUCTION

In April of 1951, L. W. Koch, of the Harrow Laboratory, observed in a greenhouse cucumber crop at Leamington, Ontario, a number of necrotic areas on the foliage of many plants. A few diseased leaves were brought back to the laboratory and were examined by the writer. Later visits to this greenhouse showed that the disease had spread rather rapidly, and finally affected a high percentage of the plants. The disease became progressively less destructive, however, as the season advanced. During June and early July of 1953, a similar outbreak of this disease was observed in another series of greenhouses near Leamington, and again a rather extensive destruction of the foliage resulted.

Trichothecium roseum (Hoffm.) Link ex Fr. was isolated from the advancing margins of the infected leaf areas on both occasions. Although this fungus did not cause a rapid destruction of the entire surface of affected leaves, similar to that brought about by certain other pathogens, the slow but progressive rotting of the leaf areas resulted in decreased yields, and consequently, was of economic importance.

This paper includes a description of the disease, and several inoculation experiments conducted under natural and controlled environmental conditions.

LITERATURE REVIEW

Ainsworth and Bisby (1) record that the type specimen of *T. roseum* was described on apple by Link in Species Plantarum in 1824. Since that time, the fungus has been reported as being saprophytic and parasitic on a large number of different plant tissues. Koch (8) reported *T. roseum* to be parasitic on the stroma of the black knot fungus, *Dibotryon morbosum* (Sch.) T. & S. It has been demonstrated that this fungus causes rots of considerable economic importance on apples, pears, bananas and oranges, and a rot following wounds on cherries, apricots and quinces (2, 3, 6, 10). Deems (4) reported that this fungus causes occasionally a fruit rot of greenhouse tomatoes in Ohio, U.S.A. Wiant (11) found it causing a fruit rot of honey-dew melons and cantaloupes. Kalashnikoff (7) reported that cucumbers grown under glass in the Leningrad area were severely attacked

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[2] Plant Pathologist.

in the early spring of 1935 by *T. roseum*. It caused a wet rot of stem, leaf and flower.

DISEASE SYMPTOMS AND CAUSAL FUNGUS

(*a*) *Symptoms*

The disease is characterized by the presence of lesions on the leaves which are at first water-soaked in appearance, and later become dry and brownish-coloured. Lesions occurring upon leaves of thin texture, such as those produced during the winter and early spring months or under conditions of high humidity, increase in size most rapidly. These lesions invariably show a relatively broad yellowish border. In old lesions of this type the centre often becomes torn and may eventually fall out. On the other hand, lesions developing upon thicker leaves produced during the late spring and early summer months show no yellowish border and the affected tissue usually dries to an ashy-brown colour and remains intact. Infected leaves vary considerably as to the number and size of the lesions. Occasionally single lesions affect as much as one-third of the entire leaf surface. More often, leaves may have as many as three or four separate infections, which later coalesce. Careful macroscopic examination of the surface of the lesion usually shows a sparse mycelial growth with a pinkish cast.

Under conditions of natural infection in commercial houses, the fungus cannot penetrate the living tissue and develop lesions without first growing for a time on decaying organic matter. For most lesions, fecal deposits of the pollinating honey bee serve as infection loci (Figure 3). Less frequently, an abscissed blossom that remains on healthy leaf tissue serves as an infection locus. Although it has been reported that this fungus causes a rot of other plant tissues, the writer has not observed any infection of cucumber tissues other than on the leaves.

(*b*) *Causal Fungus*

The causal fungus was readily identified as *Trichothecium roseum* (Hoffm.) Link ex Fr. Typical conidia produced acrogenously on simple conidiophores are shown in Figures 4 and 5.

INFECTION EXPERIMENTS

On May 10, 1951, fifty cucumber plants in the laboratory greenhouse were sprayed with an aqueous spore suspension of *T. roseum* and a moderately high humidity was maintained for the following four weeks.

By June 3, lesions had developed on the leaves of many of the inoculated plants (Figure 1). For all the lesions, bee excreta served as the infection site. Whereas relatively few lesions of small diameter were present on the topmost leaves, numerous and large ones had developed on leaves underlying the uppermost carpet of foliage.

To determine how quickly *T. roseum* would parasitize a healthy cucumber leaf after its establishment upon an appropriate saprophytic substrate the following inoculations were made. Small pieces of potato dextrose agar, on which the fungus was growing, were pressed against bee excreta on the upper surface of twenty cucumber leaves in the laboratory

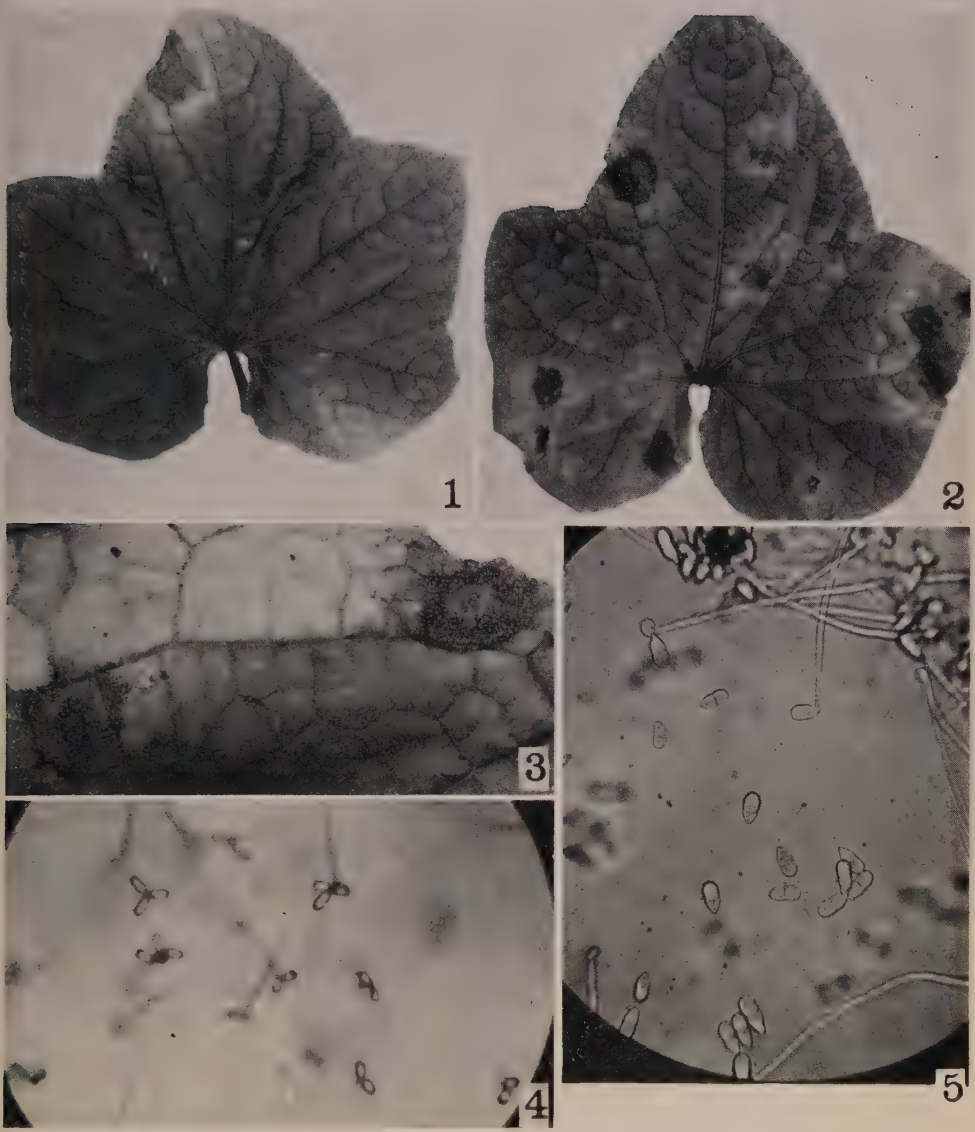

FIGURE 1. Cucumber leaf showing a lesion caused by *T. roseum* when the plants were sprayed with a suspension of conidia.

FIGURE 2. Artificially inoculated cucumber leaf, showing several infections. Photograph taken 7 days after P. D. agar overgrown with fungus was superimposed upon bee excreta.

FIGURE 3. Portion of a naturally infected cucumber leaf, showing two lesions Note bee feces in centre of each lesion.

FIGURE 4. Photomicrograph of *T. roseum.* Conidiophores bearing two or more conidia (approx. ×120).

FIGURE 5. Photomicrograph of *T. roseum* showing mature conidia (approx. ×250).

greenhouse. As a check, uninoculated pieces of agar were placed over similar sites on twelve leaves. Only leaves in partial shade were inoculated. To provide a moist atmosphere the inoculated leaves were covered with a cellophane bag.

Within seven days, water-soaked, tan-coloured areas ranging up to three-fourths of an inch in diameter had developed around 60 per cent of the excreta upon which the fungus inoculum had been placed (Figure 2). Lesions developed at approximately the same rate on young and old inoculated leaves. No lesions developed where the non-inoculated agar blocks were applied. Microscopic examination of the surface of these lesions revealed that the fungus had developed conidia in large numbers on the leaf substrate, a short distance behind the advancing margin of the lesion.

In other tests where abscissed cucumber blossoms infected with *T. roseum* were held against the upper surface of the cucumber leaf by means of a toothpick fastened at the leaf margin with a paper clip, the fungus produced typical lesions under conditions of high humidity. In the absence of high humidity the flower tissue dried rapidly and the fungus failed to penetrate the leaf tissue.

In further experiments where young, detached cucumber fruits were inoculated by spraying the attached flowers with an aqueous spore suspension of *T. roseum* and were held in a moist chamber, the fungus mycelium permeated the young fruits and caused a soft rot. However, when young unsevered fruits were sprayed with a similar spore suspension, the fungus failed to cause decay, even in a relatively humid greenhouse atmosphere. Although the flower tissues were permeated with fungus mycelium, the flowers abscissed before *T. roseum* became established upon the adjoining fruit.

DISCUSSION

A pathogen that seldom attacks a crop grown in the field may occasionally become destructive when the same crop is grown within the confines of a greenhouse. This has proved to be true for *Botrytis cinerea* Pers. on cucumbers in Ontario (9). *T. roseum* is another fungus that falls into that category, and of interest is the fact that it exhibits a type of parasitism similar to that of *B. cinerea*.

Although all the factors giving rise to an epidemic of *T. roseum* on cucumbers may not have been examined, the results of infection tests recorded above indicate that this disease is unlikely to become troublesome in the field crop for the following reasons. *First*, the dependence of the fungus upon decaying organic matter, such as insect excrement and abscissed blossoms, for its initiation of infection, is a condition that is unlikely to be fulfilled in a crop grown in the field. *Second*, the existence in the field of humidities normally much lower than those in the greenhouse would be unfavourable for the development of lesions, even if the previous requisite for infection were met.

The fact that this disease has occurred to date in only a few of the numerous greenhouses of the Leamington, Ontario, area, would indicate a local build-up of fungus inoculum and a subsequent limited spread. In this respect, *B. cinerea*, although much more prevalent than *T. roseum*, follows an essentially similar pattern. There is a great likelihood that both fungi gain admission to the greenhouse on mulching materials.

Successful control measures for this disease have not yet been satisfactorily worked out. Although the elimination of the honey bee would, to a great extent, eliminate infection loci, the insect is required for pollination and could not be sacrificed. Since high humidity is a prerequisite for infection, and also because this condition is conducive to rapid lesion development, it would seem that the disease might be effectively controlled by lowering greenhouse humidity. This procedure often involves considerable changes in cultural practices and it may bring about conditions less favourable for optimum growth of the plants with resultant lower production. Perhaps, more effective disease control could be obtained through the application of fungicides. In that connection, however, the results of spraying with ferbam, a fungicide that controls outbreaks of *B. cinerea* effectively (7), have not been promising.

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THE REACTION OF BARLEY VARIETIES TO RYE STEM RUST, *PUCCINIA GRAMINIS* VAR. *SECALIS*[1]

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[Received for publication February 8, 1954]

ABSTRACT

Indications derived from Uniform Rust Nurseries that a number of barley varieties resistant to wheat stem rust (*Puccinia graminis* var. *tritici*) were susceptible to rye stem rust (*P. graminis* var. *secalis*) were confirmed by tests made in the greenhouse and in the field. Analyses of stem-rust collections from rust-resistant barley varieties showed these, in most cases, to consist of rye stem rust. In determining the reaction of barley varieties to rye stem rust it was found that the reaction of the seedling leaf gave little indication of the reaction of the stem of the adult plant. It was therefore necessary to test plants in the adult stage to determine varietal reaction to the rust. Out of 270 barley accessions tested in this way only one showed high resistance to rye stem rust. It is concluded that while rye stem rust is not a threat to barley production at present it is a potential danger that should not be ignored.

INTRODUCTION

In North America, wheat stem rust, *Puccinia graminis* Pers. var. *tritici* Erikss. and Henn., has been the rust of greatest significance for barley and, consequently, plant breeders concerned with the production of rust-resistant barley varieties have concentrated on developing varieties resistant to it. In this plant breeding program, rye stem rust, *P. graminis* Pers. var. *secalis* Erikss. and Henn., has been largely neglected, presumably because of its relative scarcity in the barley-growing areas of the central part of the continent. That barley may be rusted by rye stem rust is well known but little attention has been given to the reaction of barley varieties to that rust. Cotter and Levine (1), who studied the physiologic specialization of rye stem rust, did not report the reaction of barley varieties to any of the 13 physiologic races found to occur in the United States and did not make any rust collections from cultivated barley. M. N. Levine did, however, report, in private correspondence with the senior author, that the barley variety Peatland had been tested in the seedling stage for its reaction to race 7 of rye stem rust and had proved susceptible to this race.

In Canada, it first became evident in 1947 and 1949 that varieties of cultivated barley were being rusted by rye stem rust. These observations would have been of little interest had it not been for the fact that some of the varieties were known to be highly resistant to wheat stem rust and, on that basis, were regarded as rust-resistant varieties. Their apparent lack of resistance to rye stem rust consequently appeared to call for further investigation. It is the purpose of the present paper to bring forward such facts as are known at present concerning the reaction to rye stem rust of barley varieties, and to discuss these facts in relation to any possible action that might be required in the future.

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DATA FROM UNIFORM RUST NURSERIES

Attention was first directed to the possibility that rye stem rust might be causing infection on rust-resistant barley varieties in 1947 when 30 per cent stem rust infection was recorded on the variety Vantage in the Uniform Rust Nursery at Fredericton, N.B. Owing to failure of the rust spores to cause infection in subsequent greenhouse tests no data were obtained as to the identity of the rust. In 1949, Vantage carried an infection of 60 per cent, and Wisc. H. 106, another supposedly stem-rust resistant variety, an infection of 40 per cent at Fredericton (Table 1). Since no rust occurred on wheat varieties but a considerable amount on adjacent plants of *Agropyron repens*, the rust was presumed to be rye stem rust. This supposition was confirmed later by infection tests that showed the rust to be pathogenic to rye varieties and to barley but not to wheat. Subsequent data from Fredericton for the years 1950, 1951 and 1952, when no wheat stem rust was present in the rust nursery, and the rye variety Prolific was included as a tester for the amount of rye stem rust, show clearly that the rust-resistant barley varieties (Vantage, Wisc. H. 106, Peatland, and U.M. 43-1020) are not appreciably more resistant to rye stem rust than the susceptible variety Montcalm. However, the data from this rust-nursery station, as well as from Kentville, N.S. in 1951, indicate that the barley varieties tend to carry a somewhat lower percentage of infection than Prolific rye.

TABLE 1.—PERCENTAGES OF STEM RUST INFECTION ON BARLEY VARIETIES IN UNIFORM RUST NURSERIES AT FREDERICTON, N.B., AND KENTVILLE, N.S.

Barley var.	Stem rust infection, in per cent							
	Fredericton, N.B.							Kentville, N.S.
	1947	1948	1949	1950	1951	1952	1953	1951
Goldfoil, C.I. 928	40	10	70	Tr	—	—	—	—
Gold, C.I. 1145	—	20	50	—	—	—	—	—
Plush, C.A.N. 1106	15	—	—	—	—	—	—	—
Montcalm, C.A.N. 1135	—	—	—	3	30	60	5	15
Vantage, C.A.N. 1162	30	15	60	Tr	5	50	3	10
Wisc. H. 106	—	—	40	2	20	60	5	10
Peatland, C.I. 5267	—	—	—	Tr	10	40	5	10
U.M. 43-1020	—	—	—	—	10	60	30	15
Prolific rye	—	—	—	30	70	70	60	50
Susceptible wheats	0	Tr	0	0	0	0	Tr	0

NOTE: — Signifies that variety was not included in test.

DISTRIBUTION OF RYE STEM RUST

Rye stem rust has not hitherto been a factor of any importance in barley production. Any threat it may assume to rust-resistant varieties will depend on its distribution, its ability to survive from year to year, and the rapidity with which it can increase on susceptible hosts. All these factors may be influenced by changes in the distribution of its host plants. An increase in the acreage devoted to rye or barley would tend to increase the amount of the rust which, in turn, would provide more opportunities for it to overwinter. Rye stem rust may overwinter, in favourable seasons, as far north as Winnipeg, Man., where Johnson and Green (2) observed overwintering on its grass host *Agropyron repens*, in the spring of 1951. The scarcity of rye stem rust in the Mississippi Valley area in the past should not be regarded as a guarantee that this rust will always be equally scarce in that region in the future.

Rye stem rust has not been of economic importance and it is only recently that attempts have been made to gain information on its distribution in Canada. Since 1950, the rye variety Prolific has been included in the Uniform Rust Nurseries as a tester for the presence of the rust, and during the same period it has been the practice to analyse all stem-rust collections from barley for the presence of both wheat stem rust and rye stem rust.

The data derived from the rust nurseries indicate that the rust is considerably more widespread in Eastern Canada (Ontario, Quebec, and the Maritime Provinces) than in the Western provinces. In 1950, it was found in 10 out of 20 nurseries in Eastern Canada but occurred in only 3 of the 14 nurseries in Western Canada (Brandon, Morden and Headingly in Manitoba). In 1951, it occurred in 13 of 20 Eastern nurseries and in 8 of 14 Western nurseries. In 1952, it was present in only 4 of 13 Western nurseries but appeared in 14 of 19 Eastern nurseries. In 1953, it was found in 6 of 13 Western nurseries but occurred in 15 of 20 Eastern nurseries.

The data from the rust nurseries is to some extent supplemented by information derived from the annual rust surveys on the occurrence of rye stem rust on barley. In 1949, in a total of 37 stem-rust collections from barley, wheat stem rust alone occurred in 23 collections, rye stem rust alone in 6 collections, and both kinds of rust together in 8 collections. In that year, rye stem rust occurred on barley collected in British Columbia, Alberta, Manitoba, Ontario, Quebec, and New Brunswick.

In 1950, rye stem rust was rarely found in collections from barley, occurring in only 3 collections (2 from Ontario and 1 from New Brunswick) out of 29 collections analysed.

In 1951, in 60 collections from barley (18 from Eastern Canada and 42 from Western Canada) 47 isolates of rye stem rust and 29 of wheat stem rust were obtained. In the 18 collections from Eastern Canada, only rye stem rust could be found. In the 42 collections from Western Canada, wheat stem rust and rye stem rust were present in about equal proportions—29 isolates of each being obtained. It is worth noting that 14 collections from the stem-rust resistant varieties Peatland, Vantage, Wisc. H. 106,

Moore and U.M. 43-1020 included only rye stem rust. The rust survey of 1951 showed also that wild barley (*Hordeum jubatum*) played a role in the propagation of rye stem rust. Twenty-two collections of stem rust from wild barley were made in the Prairie Provinces. From these were obtained 13 isolates of wheat stem rust and 12 of rye stem rust.

In 1952, rye stem rust was rarely collected on barley in Western Canada; of 58 isolates from barley only one was identified as rye stem rust. In Eastern Canada, however, rye stem rust appeared to predominate on barley, as 10 of the 13 isolates studied were rye stem rust.

In 1953, a year of heavy wheat stem rust infection in Western Canada, the figures were 5 rye-stem-rust isolates out of a total of 41 from barley, while in Eastern Canada 6 rye-stem-rust isolates were obtained from a total of 23.

As far as conclusions are possible from the data now available it appears that the prevalence of rye stem rust varies considerably from year to year, especially in the Prairie Provinces where barberry is not available as an annual source of inoculum. In that region, overwintering of the uredial stage on native grass hosts such as couch grass (*Agropyron repens*) is the most likely source of the annual inoculum of the rust. The wide distribution of the rust in 1951 is perhaps due to considerable overwintering in the spring of that year (2).

EXPERIMENTS ON THE REACTION OF BARLEY VARIETIES TO RYE STEM RUST

In determining the reaction of cereal varieties to their rusts it is common practice to infect seedling leaves and determine the reaction of the variety by the type of rust pustule formed on the leaves. When this method of study is applied to stem rust it is assumed that there is a close correlation between the type of rust pustule produced on the leaf of the seedling plant and the type of pustule formed on the stem of the adult plant. Experience has shown that for stem rust of wheat and oats this relationship is usually dependable.

In barley, the relationship between the reaction of seedling leaf and adult-plant stem is not so close as in wheat or oats but the seedling reaction is nevertheless of some value in tests for resistance to wheat stem rust. However, when seedling-leaf tests were undertaken to determine the reaction of barley varieties to rye-stem-rust cultures it was found that the leaves of the many varieties tested reacted much alike and, in general, indicated a moderate degree of resistance in sharp contrast to the susceptible reaction of the same varieties in the field. Repeated greenhouse tests have led to the conclusion that the easy and rapid seedling-leaf test for evaluating rust resistance is of little value in studies with rye stem rust.

Adult-plant Reaction in the Greenhouse

In view of the fact that race 15B of wheat stem rust is the predominant race of that rust and perhaps the race most generally pathogenic to barley it was decided to compare this race and rye stem rust for their virulence to a group of barley varieties.

The plants were grown in 6-inch flower pots, 4 plants to a pot. Seeding was done on January 14, 1952, and the plants were inoculated during the

last week of March when they were just headed. Inoculum of race 15B was composed of a number of cultures from different sources, as such a composite was considered to be more representative of the race than a single culture. For the same reason several rye-stem-rust cultures were bulked to provide inoculum of that rust. Following infection, rust development took place at ordinary greenhouse temperatures which ranged daily from a low of about 60° F. at night to a high of about 85° F. in early afternoon.

The data derived from this test of 19 barley varieties are recorded in Table 2 and a comparison with the seedling reaction is made. In considering the data, the following comments seem permissible:

(1) In both tests, the adult leaf was somewhat more resistant than the seedling.

(2) In both tests, the stem was more susceptible than the seedling.

(3) In both tests, the stems were considerably more susceptible than the leaves.

(4) On the whole, the varieties tested were somewhat more susceptible to rye stem rust than to wheat-stem-rust race 15B. If the infection type x+ indicates moderate susceptibility and types 3 and 4 full susceptibility, then 11 of the 19 varieties were susceptible to race 15B and 15 of the 19 varieties were susceptible to rye stem rust. If types x, (×)−, (×)=, etc., indicate different degrees of resistance, then 8 varieties showed more or less resistance to race 15B while only 4 varieties showed resistance to rye stem rust.

TABLE 2.—REACTION OF BARLEY VARIETIES TO RACE 15B AND TO RYE STEM RUST

Variety	Mature plant reaction								Seedling reaction	
	Race 15B				Rye stem rust				Pustule type	
	Stems		Leaves		Stems		Leaves			
	Pustule type	%	Pustule type	%	Pustule type	%	Pustule type	%	15B	Rye stem rust
U.M. 1020	(x)+	40	(;)(1)	2	(3)(4)C	10	(1)	tr	(2)(3)+	(2)(3)−
U.M. 348	(x)	25	(x)−	3	(x)+	40	(x)−	3	(3)	(3)−
Fjola	(4)+	60	(1)(2)+	10	(4)	40	(1)(2)+	5	(2)(3)−	(2)(3)−
S.C. 235	(4)−	30	(1)(2)+	15	(x)	25	(1)	tr	(;)(2)+	(;)(2)+
H.106.1	(x)	45	(;)(1)	tr	(3)(4)	40	(1)(2)	tr+	(1)(2)−	(;)(1)+
C.I. 6614	(x)+	40	(1)(2)	5	(3)(4)	25	(1)(2)	tr+	(1)(2)+	(1)(2)
C.I. 6613	(x)	20	(;)(1)	tr	(3)(4)	45	(1)(2)	5	(2)(3)−	(1)(3)−
Valentine	(x)=	5	(;)(1)−	tr	(x)−	5	(1)−	tr−	(1)(2)+	(1)(2)+
Vantage	(;)(1)(2)	tr+	(;)(1)	tr	(x)+	25	(1)	tr+	(1)(2)	(1)(2)
Peatland	(x)−	20	(;)(1)	2	(3)(4)	35	(1)	tr+	(2)(3)−	(2)(3)−
Feebar	(x)+	25	(;)(1)	tr+	(x)+	8	(1)(2)	tr	(1)(2)−	(1)(3)
Pillsbury	(x)+	30	(;)(1)+	2	(x)	5	(1)(2)	tr	(2)(3)	(1)(3)
Jet	(3)(4)	45	(;)(1)	tr+	(x)+	25	(1)±	tr+	(1)(2)+	(1)
Valkie	(3)(4)	35	(1)(2)+	10	(3)(4)−	15	(1)	tr	(1)(3)	(1)(2)+
Moore	(x)	40	(1)(2)	1	(x)+	35	(1)	tr	(1)(3)	(1)(3)
Titan	(4)	60	(1)(2)+	10	(4)C	35	(1)(2)	1	(2)(3)	(x)−
Montcalm	(x)+	50	(1)(2)+	2	(3)(4)C	40	(x)	5	(3)±	(3)±
Harlan	(4)	60	(1)(2)+	5	(x)++	20	(1)(2)	tr	(2)(3)+	(2)(3)−
Chevron	(x)	10	(;)(1)	tr	(x)	10	(;)(1)	tr	(1)(2)	(1)(2)

Explanation of symbols for pustule types: (;) near-immunity; (1) high resistance; (2) and (x) =moderate resistance; (x)− and (x) indeterminate reaction expressing slight resistance; (x)+ and (3) moderate susceptibility; (4) complete susceptibility.

(5) Of the varieties tested it appears that Vantage and Valentine were the most resistant to race 15B and that Valentine was the most resistant to rye stem rust.

Field Test of Reaction to Race 15B and Rye Stem Rust

The above greenhouse test was followed up by field tests, at Winnipeg, on the reaction of 32 barley varieties to wheat-stem-rust race 15B and rye stem rust. The same composite rust samples were used as in the greenhouse test.

In these field studies, attempts were made in separate tests to produce infection by race 15B and by rye stem rust with a minimum of contamination by other rusts. To make this possible the two plots were separated from one another by a distance of about a half-mile, and the rye-stem-rust plot was located at least that far from any other artificially produced stem-rust infection.

The plots were sown early so that the experiments might be completed before the onset of much natural infection, and the two plots were subjected to infection by the respective rusts on June 17. Border rows for rust increase were composed of Prolific rye for the rye-stem-rust plot and of a mixture of Lee and Nugget wheats for the plot inoculated by race 15B. Notes taken on the amount of infection just before the end of July are presented in Table 3.

The objective of avoiding extraneous infection in the test plots was only partially achieved. The 15B-test plot was almost free of rye stem rust. The rye-stem-rust plot showed, however, a light infection of wheat stem rust at the time notes were taken. The varieties Lee, Garnet, and Marquis were used as indicators of the amount of wheat stem rust present, 4 rows of each being included (only 2 of each are shown in Table 3). The average infection was trace for Lee, 16.2 per cent for Garnet and 2.0 per cent for Marquis. Nevertheless it was clear from the heavy infection on the check rows of Prolific rye and from analysis of the rust from some of the barley varieties that the infection in this plot was predominantly caused by rye stem rust.

In the 15B test there was a sharp distinction between resistant and susceptible varieties. Valentine, Vantage, Peatland, Feebar, Pillsbury, and Chevron showed only traces of infection with a resistant or moderately resistant reaction, whereas many other varieties showed infection of from 25 per cent to 60 per cent and a susceptible reaction. No such sharp distinction could be detected in the rye-stem-rust test. The percentage infection varied greatly from variety to variety but the reaction appeared to be mostly of a susceptible type. As the experiment was not replicated, it is doubtful if much significance should be attached to the differences in the amount of infection on different varieties. It may be noted, however, that the variety Valentine, which seemed the most resistant one in the greenhouse test, bore one of the lowest percentages of infection in the field test, and that the variety Black Hulless showed both a low percentage infection and a moderately resistant reaction.

TABLE 3.—PERCENTAGE OF INFECTION IN FIELD PLOTS, AT WINNIPEG, IN 1952, ON BARLEY VARIETIES EXPOSED TO (1) RYE STEM RUST, AND (2) RACE 15B OF WHEAT STEM RUST

Variety	Rye stem rust test %-reaction		Race 15B test %-reaction	
Check—Lee wheat	tr−		30	
Check—Garnet wheat	10		—	
Check—Marquis wheat	tr+		—	
Check—Prolific rye	25		tr	
U.M. 43-1020	20	S	3	MR
U.M. 348	25	S	15	S
Fjola	25	S	50	S
S.C. 235	30	S	20	S
Wisc. H 106.1	15	S	tr	R
C.I. 6614	20	S	5	MR
C.I. 6613	30	S	5	MR
Valentine	5	S	tr+	R
Vantage	25	S	tr	R
Peatland	20	S	tr+	MR
Feebar	5	S	tr	MR
Pillsbury	10	S	tr+	MR
Jet	15	S	20	S
Valkie	5	S	25	S
Moore	20	S	5	MR
Titan	35	S	50	S
Montcalm	20	S	35	S
Harlan	35	S	50	S
Chevron	15	S	tr	R
Atlas	20	S	40	S
Baku	5	S	20	S
Bay Brewing	25	S	60	S
Black Barbless	20	S	30	S
Black Hulless	3	MR	15	S
Icelandic	20	S	5	MR
Stevens—46	15	S	3	MR
Stevens—41	15	S	5	MR
Heitpas—3	10	S	10	MR
Heitpas—5	5	S	10	S
Bon Rudin—5	10	S	5	S
Zander—1	20	S	15	MR
Ricardo	10	S	35	S
Check—Prolific rye	50		tr	
Check—Lee wheat	tr		40	
Check—Garnet wheat	25		—	
Check—Marquis wheat	5		—	
	Notes July 28		Notes July 31	

NOTE: Symbols for reaction: R = resistant; MR = moderately resistant; S = susceptible.

Reaction, in the Greenhouse, of 270 Barley Accessions to Rye Stem Rust

Because of the difficulties experienced in the preceding experiment in obtaining field reaction of varieties to rye stem rust in the absence of infection by wheat stem rust it was decided to test a large number of barley varieties for their reaction in the greenhouse. Accordingly, 270 accessions were tested in both seedling and adult stages in the greenhouse. The rust used was culture No. 285-52 collected on Vantage barley at Fredericton, N.B., and identified as rye stem rust. The barley varieties were tested in the seedling stage in November and December, 1952, at a temperature generally maintained within the range of 60° to 65° F. All the varieties were rather

highly resistant as judged by the reaction of the seedling leaf. The reactions, however, were not identical for all varieties. Infections ranged from necrotic flecks on some varieties to 2— type of pustule on others.

For the adult-plant tests, the same accessions were planted at the beginning of May, 1953 in rows about 2 feet long in seed beds in the greenhouse. On July 6, when the earlier-maturing varieties were in the heading stage, all the plants in the seedbeds were inoculated with the above-mentioned source of rye stem rust. Later-maturing varieties, which were not in head at the time of inoculation, were re-inoculated on August 2.

The accessions were classified as resistant, moderately resistant, moderately susceptible, and susceptible on the basis of the type of rust pustule formed on the stems. In this classification, pustule types 1 to 2 were regarded as indications of resistance, types 2 to 3— of moderate resistance, type 3 of moderate susceptibility, and type 3+ to 4 of susceptibility. The varieties tested are enumerated below in accordance with this classification.

The only accession in the whole test that showed reasonably high degree of resistance was Black Hulless C.I. 666. It may be mentioned here that this variety was the only one that showed any degree of resistance to rye stem rust in the field test of 1952 (see Table 3). That this variety does not possess much resistance to race 15B of wheat stem rust is indicated by the fact that in the 15B field test it carried 15 per cent infection of a susceptible type.

Adult-plant Reaction of 270 Barley Accessions

Resistant:

Black Hulless C.I. 666.

Moderately resistant:

German Brewing C.A.N. 1008; Hanna C.I. 1122; *Hordeum deficiens decorticatum* C.I. 2230.

Moderately susceptible:

Anoidium C.I. 7269; Austral C.I. 6483; Bay Brewing C.I. 257; Beecher C.I. 6566; Beldi C.I. 190; C.I. 1961; C.I. 3737; C.I. 5644; C.I. 5647; Compana C.A.N. 1154; Dorsett C.I. 4821; Foreign 127 C.A.N. 48; Gatami C.I. 575; Gatami C.I. 2276; Gold C.I. 1145; Goldfoil C.I. 928; Halikon C.A.N. 52; Halikon C.I. 6004; Hanna R.L. 114; Hero C.I. 4602; *H. distichon nigrum* C.A.N. 21; *H. distichon nudum* C.A.N. 22; *H. distichon nutans* A C.A.N. 24; *H. intermedium cornutum* C.I. 2215; *H. vulgare atrum* C.I. 2204; Horsford C.I. 1775; Italy C.I. 914; Montcalm C.I. 7149; Morocco 077 C.A.N. 1131; Persicum C.I. 6531; Prospect C.I. 6339; S.C. 235 R.L. 279; Smyrna C.I. 910; Spartan C.I. 5027; Texan C.I. 6499; Warrior C.I. 6991; White Gatami C.I. 920.

Susceptible:

Abacus C.I. 1088; Abyssinia C.I. 2192; Acc. no. 814 B.40 R.L. 13; Acc. no. 817 B.44 R.L. 14; Afghan C.I. 6366; Algerian C.I. 1179; Alpha C.I. 959; Archer C.I. 1031; Archer Goldthorpe C.A.N. 1003; Arequipa C.I. 2329; Arlington Awnless C.I. 702; Atlas C.I. 4118; Austrian Hanna St. 66 C.A.N. 46; Baker C.I. 975; Baku C.I. 709; Barboff C.I. 7148; Bark C.I. 2793; Batna C.I. 3391; Bavaria C.I. 6395; Beldi Giant C.I. 2777; Black Arabia C.I. 202; Black Barbless C.A.N. 11; Blackhull C.I. 878; Blackhull C.A.N. 813; Black Hull-less (Lennoxville) C.I. 1097; Blue Hulless C.I. 4848; Bolivia C.I. 1257; Byng C.I. 6089; Cal. Brewing C.I. 4870; California feed C.I. 799; Cal. Mariout C.I. 1455; Callas C.I. 2440; Can. Thorpe C.I. 740; Cape C.I. 557; Carre 26 C.I. 3386; Carre 180 C.I. 3390; Cebada Capa C.I. 6193; Charlottetown 80 C.I. 2732; Chevalier C.I. 278; Chevron C.I. 1111; Chile R.L. 49; Chilean D. C.I. 1433; Chillian Brewing C.I. 657; Clifford C.I. 1910; Club Mariout C.I. 261; Coast C.I. 276; Coast C.I. 690; Colsess C.I.

2792; Cruzat C.I. 6482; C.I. 510; C.I. 1347; C.I. 1613; C.I. 2223; C.I. 2237; C.I. 2329; C.I. 2492; C.I. 2538; C.I. 2542; C.I. 2549; C.I. 4156-2; C.I. 4160-1; C.I. 4219; C.I. 4220-1; C.I. 4223-2; C.I. 4356; C.I. 4974; C.I. 4975; C.I. 4979; C.I. 5324; C.I. 5326; C.I. 5366; C.I. 5862; C.I. 5863; C.I. 6306; C.I. 6915; Danish Island C.A.N. 1002; Danubian C.I. 6525; Duckbill C.I. 1916; Egypt 4 C.I. 6481; Egyptian Sudan C.I. 6489; Featherston C.I. 1120; Feebar C.I. 7620; Foreign 82B C.A.N. 62; Frankonia C.I. 680; French Chevalier C.I. 175; Galore R.L. 99; Gartons C.I. 645; Gem C.I. 7243; Glabron C.I. 4577; Glacier C.I. 6976; Golden Drop C.I. 2135; Golden Pheasant C.I. 2488; Gordon C.I. 4842; H.106.1 R.L. 278; Hanna C.I. 203; Hanna C.I. 906; Hannchen C.I. 531; Heil's Hanna C.I. 682; Hero C.I. 1286; Heys Special C.I. 6487; Himalaya C.I. 620; Hooded Spring C.I. 716; *H. deficiens deficiens* C.I. 2225; *H. deficiens steudelli* C.I. 2226; *H. deficiens triceros* C.I. 2227; *H. deficiens tridan* C.I. 2228; *H. distichon angustispicatum* C.I. 2219; *H. distichon nigrilaxum* C.I. 2224; *H. distichon nigrinudum* C.I. 2222; *H. distichon nutans* C.A.N. 23; *H. distichon nutans* B C.A.N. 25; *H. distichon rimpaui* C.I. 2220; *H. hexastichum eurylepsis* R.L. 140; *H. hexastichum pyramidatum* R.L. 141; *H. intermedium mortoni* C.I. 2210; *H. intermedium nudihaxtoni* C.I. 2213; *H. intermedium nudimortoni* C.I. 2214; *H. tetrastichum coerulescens* R.L. 150; *H. tetrastichum pallidum* R.L. 146; *H. tetrastichum pallidum* R.L. 147; *H. tetrastichum pallidum* R.L. 148; *H. tetrastichum pallidum* R.L. 149; *H. vulgare aethiops* C.I. 2208; *H. vulgare horsfordianum* C.I. 2203; *H. vulgare trifuscatum* C.I. 2207; Horn C.I. 926; Horsford C.I. 507; Horsford C.I. 877; Icelandic C.A.N. 90; Jet C.I. 967; Juliaca C.I. 1114; Kindred C.I. 6969; Korsbyg C.I. 918; Kuba Summer C.I. 6480; Kwan C.I. 1016; Lechtaler C.I. 6488; Luth C.I. 972; Mainwali C.I. 3400; Malting C.I. 1129; Manchuria C.I. 2330; Manchurian C.I. 739; Mansfield C.I. 2241; Mariout B113 C.A.N. 91; Mariout B C.A.N. 1130; Mecknos Morocco C.I. 1379; Mensury C.I. 4696; Michigan Black C.I. 923; Michigan C.I. 2782; Michigan 110 R.L. 177; Minsturdi C.I. 1556; Modia C.I. 2483; Morocco C.I. 3902.1; Morocco C.I. 6311; Nepal C.I. 595; Newal C.I. 6088; Nobarb C.A.N. 1143; O.A.C. 21 C.I. 1470; Odessa C.I. 182; Oderbrucker C.I. 940; Oderbrucker C.I. 4666; Oderbrucker C.I. 957; Olli C.I. 6251; Orge B.100 C.A.N. 30; Orge 14 B.101 C.A.N. 31; Orge Forrager B.102 R.L. 198; Oregon C.I. 4871; Orel C.A.N. 14; Orel R.L. 194; Pamella Blue C.I. 3609; Pannier C.I. 1330; Paso C.I. 5047; Pearl C.I. 4834; Peatland C.I. 5267; Peru C.I. 2302; Peruvian C.I. 935; Peruvian Sel. 1. C.I. 5912; Peruvian Sel. 19. C.I. 6568; Plumage Archer C.I. 5033; Plush C.I. 7030; Polish C.A.N. 56; Pontiac C.I. 4849; Princess C.I. 529; Prussian C.A.N. 58; Purple Nepal C.I. 2242; Psaknon B.81 C.A.N. 34; Quinn C.I. 1024; Recha 1. C.I. 5051; Regal C.I. 5030; Rex C.I. 1388; Sacramento C.I. 4180; Sahara C.I. 3770; Sanalta C.I. 6087; Sandred C.I. 937; S.C. 235 R.L. 279; Scotch Standwell C.A.N. 1007; Silver King C.I. 890; Smooth Awn × Manchuria 11-20-10 R.L. 232; Smooth Awn × Manchuria 11-21-15 R.L. 233; Smooth Awn × Manchuria 11-21-18 R.L. 234; Smyrna C.I. 195; Star C.I. 1701; Stavropol C.I. 2103; Steigum C.I. 907; Stella C.I. 2678; Stephen C.A.N. 1142; Success C.I. 4840; Sulu C.I. 1022; Svalof C.A.N. 59; Svalof Victory C.I. 5077; Svansota C.I. 1907; Swan Neck R.L. 246; Swanhak C.I. 187; Tico C.I. 6279; Titan C.A.N. 1118; Trebi C.I. 936; Tregal C.A.N. 1150; Valentine C.I. 7242; Valkie C.I. 5748; Vantage C.A.N. 1162; Vaughn C.I. 1367; Velvet C.I. 4252; Velvon C.I. 6109; Velvon 11 C.A.N. 99; Virginia Hooded C.I. 2290; Wheelers Thorpe C.A.N. 60; White Hulless C.A.N. 785; White Smyrna C.I. 2084; Wisconsin 38 C.I. 5105; 36-AB-6127 (Harlan) C.I. 7008.

DISCUSSION

Admittedly, rye stem rust is not a present threat in barley production and there is no certainty that it ever will be. It is, however, a potential menace and, as such, should not be entirely ignored.

The experiments here reported indicate that resistance to rye stem rust (or at least to the rye stem rust that has been affecting barley varieties in Canada) is not present in many barley varieties, and is largely absent from those that have resistance to wheat stem rust. If economically useful barley varieties with resistance to rye stem rust are to be produced a more intensive search will have to be made for sources of resistance than has been done thus far. If adequate sources of resistance can be found it would be desirable to institute a breeding program with the object of combining in one variety resistance to wheat stem rust and rye stem rust.

It is doubtful if such a breeding program could be set up on the basis of present knowledge. It is true that there are available a number of barley varieties with rather high resistance to wheat stem rust. Knowledge on sources of resistance to rye stem rust is much more limited. It is not even certain that the one variety, Black Hulless C.I. 666, found to be resistant in the present study, is actually resistant to all specimens of rye stem rust. It is definitely necessary to do further extensive testing of varieties to determine which ones are resistant. An obstacle in such testing work is the aforementioned difficulty of determining varietal reactions by means of seedling tests carried out in the greenhouse. Although the testing of adult plants in the greenhouse does produce more reliable results it will be generally agreed that the most reliable data obtainable are likely to come from varietal tests in field plots. To be effective, such tests would have to be carried out in some place where rye-stem-rust infection could be secured in the virtual absence of wheat stem rust.

ACKNOWLEDGMENT

The writers are indebted to John Bonk, of the Cereal Breeding Laboratory, Winnipeg, for supervising the greenhouse experiment on the testing of 270 barley accessions for their reaction to rye stem rust in the seedling and adult stages.

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EXPERIMENTS ON THE CHEMICAL CONTROL OF THE SWEETCLOVER WEEVIL, *SITONA CYLINDRICOLLIS* FAHR. (COLEOPTERA: CURCULIONIDAE)[1]

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[Received for publication February 24, 1954]

ABSTRACT

For the control of the sweetclover weevil, *Sitona cylindricollis* Fahr., in Manitoba, dieldrin, parathion, Metacide, and EPN (ethyl p-nitrophenyl thiobenzenephosphonate), each applied at half a pound per acre, and DDT, chlordane, and heptachlor at higher rates of application were effective. Dieldrin and its stereoisomer endrin were more effective than aldrin or isodrin. Applications of parathion, DDT, chlordane, or toxaphene against ovipositing adults in the spring did not significantly reduce larval populations.

INTRODUCTION

The use of DDT for the control of the sweetclover weevil, *Sitona cylindricollis* Fahr., was suggested by the work of Bird (2) and Munro *et al.* (4). Wilson (5) has shown that DDT, parathion, and toxaphene appreciably reduce populations of the weevil, and that two weeks after treatment foliage injury by the weevils was significantly reduced by applications of DDT, toxaphene, parathion, or EPN. Herron (3) reported that heptachlor and particularly aldrin were more effective than chlordane or DDT in the prevention of plant injury over a period of three weeks.

This is a report on studies, from 1948 to 1952, on the effectiveness of organic insecticides for the control of the sweetclover weevil and on the effect of sprays applied in early spring to kill ovipositing adults and reduce larval infestations.

METHODS AND MATERIALS

The toxicities of the insecticides were compared in late summer when weevils had concentrated in large numbers along the edges of first-year clover fields. This ensured accurate counts of mortality.

In 1949, two series of tests were conducted at Portage la Prairie, Man., the first from August 24 to September 3, the second from August 30 to September 9. In 1951, two series were conducted at the same location, from September 7 to 17 and from September 12 to 22. In 1952, one series was conducted at Morden, Man., from August 25 to September 4, and another at Portage la Prairie, from September 9 to 19. Weekly mean temperatures ranged from 53° to 64° F., precipitation from 0.26 to 0.55 inch.

Three randomized blocks, each consisting of a plot for each of the treatments and one untreated plot, were arranged along the edge of a sweet clover field. The plots, one-fortieth of an acre (40 by 28 feet) in area, were set up across the line of defoliation and separated from each other by a 10-foot untreated, buffer strip.

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Insecticides tested were the organic phosphates parathion, Metacide, and EPN (ethyl p-nitrophenyl thiobenzenephosphonate), and the hydrocarbons DDT, methoxychlor, chlordane, heptachlor, BHC, toxaphene, aldrin, isodrin, dieldrin, and endrin. The rates of application and formulations used are shown in relevant tables.

The sprays were applied with a gasoline-powered paint sprayer, fitted with a Lincoln oil spray gun.† Thorough coverage of all sweet clover foliage was ensured by applying the sprays at 80 gallons per acre, at a pressure of 50 pounds.

The percentage mortalities were determined on all plots 10 days after treatment by collecting the adults from 4 samples (each half a square foot) of surface soil. Percentage mortalities were corrected by Abbott's formula (1).

In 1951, at Codette, Sask., sprays were applied early in the spring in an attempt to kill ovipositing adults and reduce the resulting larval population. Duplicate 8-acre plots were sprayed on May 18 and 28, with a low-volume, low-pressure sprayer. Each spray of DDT and toxaphene

† Model 825, Lincoln Engineering Co., St. Louis, Mo.

TABLE 1.—PERCENTAGE MORTALITIES OF THE SWEETCLOVER WEEVIL 10 DAYS AFTER APPLICATION OF VARIOUS SPRAYS, FROM 4 HALF-SQUARE-FOOT SAMPLES FROM EACH OF THREE PLOTS, PORTAGE LA PRAIRIE, MAN., 1949.

Insecticide and formulation	Pounds of toxicant per acre		Total number of weevils	Per cent mortality*
	Series 1	Series 2		
Parathion[1]	0.5		577	77
15% wettable powder	0.25		556	43
DDT[2]		2.0	657	73
50% wettable powder	1.0		275	21
Chlordane[3]		2.0	411	61
40% wettable powder	1.0		220	7
BHC[4]		2.0 (gamma isomer)	556	19
6% wettable powder				
Toxaphene[5]		2.0	610	17
20% wettable powder	1.0		270	4
Methoxychlor[6]	1.0		239	8
50% wettable powder				

* Corrected by Abbott's (1) formula; mortalities in the checks did not exceed 2.2 per cent. Difference necessary for significance at 1 per cent level: 24; at 5 per cent level: 13.

[1] Thiophos. North American Cyanamid Ltd., Toronto, Ont.
[2] Micronized. "Green Cross" Insecticides, Montreal, Que.
[3] Dowchlor. Dow Chemical Co., Midland, Mich.
[4] Benexane 50. Canadian Industries Ltd., Montreal, Que.
[5] Hercules Powder Co. Inc., Wilmington, Del.
[6] Marlate. Canadian Industries Ltd., Montreal, Que.

was applied at 2.0 pounds per acre, chlordane at 1.7 pounds, and parathion at half a pound. The results were evaluated 50 days after the last spray by counting the larvae in 20 soil samples, 4 by 4 by 8 inches, taken from each plot.

RESULTS

Tests on Adult Mortalities

Table 1 shows that there was no significant difference at the 5 per cent level between the mortalities obtained with half a pound of parathion and 2.0 pounds of DDT. Mortality obtained with chlordane was similar to that recorded for DDT at 2 pounds but was lower than that for parathion at half a pound. Methoxychlor, toxaphene, and the gamma isomer of BHC were relatively non-effective at the dosage levels used.

Table 2 shows that the mortalities for half a pound of parathion and EPN and 1.0 pound of Metacide, dieldrin, and aldrin were not significantly different from those for 2.0 pounds of DDT.

Table 3 shows that aldrin and its stereoisomer isodrin are less toxic to the sweet clover weevil than dieldrin or endrin. Dieldrin at half a pound was similar to DDT at $1\frac{1}{2}$ pounds. At one pound, heptachlor was as effective as DDT. In series 2, mortalities of 75 and 79 per cent were

TABLE 2.—PERCENTAGE MORTALITIES OF THE SWEETCLOVER WEEVIL 10 DAYS AFTER APPLICATION OF VARIOUS SPRAYS, FROM 4 HALF-SQUARE-FOOT SAMPLES FROM EACH OF THREE PLOTS, PORTAGE LA PRAIRIE, MAN., 1951

Insecticide and formulation	Pounds of toxicant per acre		Total number of weevils	Per cent mortality*
	Series 1	Series 2		
Parathion 15% wettable powder	0.5		524	87
Metacide[7] 30.7% emulsion concentrate	1.0	0.25	587 525	94 37
EPN[8] 27% wettable powder	0.5	0.25	411 496	78 73
DDT 50% wettable powder		2.0	320	90
Dieldrin[9] 25% wettable powder		1.0	418	89
Aldrin[10] 25% wettable powder		1.0	200	66

*Corrected by Abbott's (1) formula; mortalities in the checks did not exceed 3.8 per cent. Difference not significant at 5 per cent level.

Spray materials as in Table 1, except:

[7] Pittsburgh Agricultural Chemical Co., New York, N.Y.

[8] E. I. du Pont de Nemours Co., Wilmington, Del.

[9] Julius Hyman & Co., Denver, Colo.

[10] Julius Hyman & Co., Denver, Colo.

TABLE 3.—PERCENTAGE MORTALITIES OF THE SWEETCLOVER WEEVIL 10 DAYS AFTER APPLICATION OF VARIOUS SPRAYS, FROM 4 HALF-SQUARE-FOOT SAMPLES FROM EACH OF THREE PLOTS, MORDEN AND PORTAGE LA PRAIRIE, MAN., 1952

Insecticide and formulation	Pounds of toxicant per acre		Total number of weevils	Per cent mortality*
	Series 1	Series 2		
Metacide		0.5	2216	75
		0.25	1967	79
30.7% emulsion concentrate	0.12		2958	50
DDT				
50% wettable powder	1.5		2458	78
25% emulsion concentrate[11]		1.5	882	77
Dieldrin				
25% wettable powder	0.5		1756	80
18.5% emulsion concentrate		0.5	1037	72
Heptachlor[12]				
25% wettable powder	1.0		2419	80
25% emulsion concentrate		1.0	900	72
		0.5	1180	51
Endrin[13]				
18.5% emulsion concentrate		0.5	1045	64
Aldrin				
25% wettable powder	0.5		2192	46
23% emulsion concentrate		0.5	794	46
Isodrin[14]				
25% wettable powder	0.5		2887	43
18.5% emulsion concentrate		0.5	1080	45

* Corrected by Abbott's (1) formula; mortality in the checks 3.5 per cent in Series 1, 14 per cent in Series 2. Difference necessary for significance at 1 per cent level: 10; at 5 per cent level: 5;

Spray materials as in Tables 1 and 2, except:

[11] Canadian Industries Ltd., Montreal, Que.

[12] Velsicol Corp., Chicago, Ill.

[13] Julius Hyman & Co., Denver, Colo.

[14] Julius Hyman & Co., Denver, Colo.

obtained with one-half and one-quarter of a pound of Metacide, respectively, although in 1951, at the lower rate, mortality was only 37 per cent. Wettable powders of DDT, aldrin, isodrin, dieldrin, and heptachlor gave essentially the same results as emulsion concentrates of these insecticides.

Tests on the Reduction of Larval Populations

The average percentage reductions in larval populations from the sprays applied in the spring were: chlordane, 46; parathion, 32; DDT, 26; and toxaphene, 14.

DISCUSSION AND CONCLUSIONS

DDT, which was included in all the experiments, gave reasonably consistent results at 1.5 or 2.0 pounds per acre, and provided a standard for the evaluation of treatments. Among the hydrocarbon insecticides, the following gave equivalent mortalities: 1.5 pounds of DDT, 1.0 pound of heptachlor, and half a pound of dieldrin. At the two-pound rate, chlordane was similar in effectiveness to DDT. The organic phosphate insecticides parathion, Metacide, and EPN, at half a pound, were as effective as 2.0 pounds of DDT. The following insecticides gave significantly lower mortalities or inconsistent results: toxaphene and BHC at 2.0 pounds; DDT, methoxychlor, and chlordane at 1.0 pound; isodrin and endrin at half a pound; and Metacide at one-quarter of a pound.

The chlorinated hydrocarbon insecticides found effective for sweet clover weevil control in Manitoba are dieldrin, heptachlor, and DDT; chlordane is probably of value but must be applied at a rate higher than DDT. DDT and chlordane are known to act slowly, but the residual action of DDT markedly increases control. The organic phosphate insecticides parathion, Metacide, and EPN give high mortalities rapidly at low dosages.

In 1952, wettable powder and emulsion concentrate formulations gave similar results.

No relationship was observed between the toxicities of the insecticides and the weather conditions that prevailed during the experiments.

Sweetclover weevil populations cannot be readily reduced by applying insecticides in the spring, for the best treatment reduced the number of larvae per sample only from 38.5 to 21, which would have allowed an adult population of 190 weevils per square foot.

ACKNOWLEDGMENTS

The writers wish to acknowledge the assistance of W. L. Askew, J. C. Guppy and C. F. Barrett, of the Brandon Laboratory. They are indebted to H. McMahon, Field Crop Insect Section, Entomology Laboratory, Saskatoon, Sask., for co-operation at Codette, Sask.

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PATHOLOGICAL HISTOLOGY OF BORON-DEFICIENT ALFALFA STEMS AND LEAVES AS AN AID IN DIAGNOSING ALFALFA YELLOWS[1]

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[Received for publication February 18, 1954]

ABSTRACT

The histopathology of boron deficiency was studied in alfalfa plants grown under field conditions and in the greenhouse in pure quartz sand cultures. The most characteristic features of boron deficiency appear in the vascular bundles near the growing tips. These features are: (*a*) the absence of thin-walled and unlignified xylem vessels; (*b*) hyperplasia and hypertrophy of cambium and phloem tissues; and (*c*) the disintegration of the cambium and phloem in extreme cases. It is believed that these abnormalities are specific to boron deficiency and constitute a valid criterion for the diagnosis of alfalfa yellows, especially whenever visual symptoms are insufficient for diagnosis.

INTRODUCTION

During the course of plant disease surveys, conducted jointly in the Lower St. Lawrence district by the Science Service Laboratory and the Experimental Station at Ste-Anne-de-la-Pocatière, it was noticed that alfalfa yellows, caused by a boron deficiency, occurred more frequently than had been realized. Moreover, symptoms quite characteristic of boron deficiency were observed on certain soils whose boron content is usually satisfactory. These symptoms could not be confused with any other known trouble, such as leaf hopper injury, etc.

The difficulties encountered in making a correct diagnosis of boron deficiency, under field conditions, led to the investigations reported in the present paper. In addition to a study of the histopathological anatomy of boron-deficient and normal legumes grown in the field as well as in the greenhouse, the possibility of using soil and plant tissue analysis as an additional or alternative criterion for detecting boron deficiency in alfalfa under Lower St. Lawrence conditions was also investigated. A field experiment designed to ascertain the boron requirements for production of alfalfa hay and seed was included in these studies. This experiment supplemented the information obtained from greenhouse work and from clinical observations in the field.

The present paper deals with comparative studies on the histology of healthy and boron-deficient alfalfa grown in the greenhouses and collected during the surveys. Similar studies on red clover and alsike clover will be considered in a later paper together with the results of chemical analyses and field work.

As far as the authors are aware, no histological study of boron-deficient alfalfa has been reported previously, although other members of the legume family have been studied. Histological investigations were made on boron-

[1] Joint contribution from the Botany and Plant Pathology Division, Science Service—Contribution No. 1375—and the Experimental Farms Service, Department of Agriculture, Ottawa, Canada.

[2] Plant pathologist.

[3] Soil specialist.

deficient broad bean by Warington (14) in 1926 and by Brenchley and Thornton (4) in 1925, while Sommer and Sorokin (10) restricted their attention to root tips of *Pisum sativum*. Brenchley (3) reported that in the absence of boron certain plants, especially many of the leguminosae, die back from the apices while anatomical changes, disintegration and blackening occur in the tissues.

MATERIALS AND METHODS

Sand cultures were set up in the greenhouses of the Experimental Station. In the late fall of 1951, alfalfa seed was sown in flats of pure quartz sand and seedlings were transplanted when they were two inches high into 2-gallon glazed crocks filled with pure quartz sand.

Colorimetric determinations by the quinalizarin method (2) revealed that the sand contained traces of boron that could not be determined quantitatively, i.e., less than 0.005 p.p.m.

Three plants were placed in each crock and each treatment was replicated five times. The composition of the nutrient solution is described in Table 1. This solution was supplied to the plants once a week. Plants were watered daily with distilled water according to needs.

Twenty-four hours before feeding, the pots were thoroughly flushed with distilled water in order to prevent accumulation of staling products. Boron, in boric acid form, was added to separate portions of the nutrient solution, so as to provide 0.0, 0.01, 0.1, 0.25, 0.50, 0.75, 1.0 and 2.0 p.p.m. of the element.

TABLE 1.—COMPOSITION OF THE NUTRIENT SOLUTION USED

Element	p.p.m. in solution	Form or forms added	
K	198	Potassium dihydrogen phosphate	KH_2PO_4
P	145	Potassium dihydrogen phosphate	KH_2PO_4
Ca	183	Calcium nitrate	$Ca(NO_3)_2 . 4H_2O$
N	124	Calcium nitrate and ammonium nitrate	$Ca(NO_3)_2 . 4H_2O$ and NH_4NO_3
Mg	49	Magnesium sulphate	$MgSO_4 . 7H_2O$
S	65	Magnesium sulphate	$MgSO_4 . 7H_2O$
Fe	15	Ferric citrate	$FeC_6H_5O_7 . 3H_2O$
Mn	0.5	Manganese chloride	$MnCl_2 . 4H_2O$
Cu	0.1	Copper chloride	$CuCl_2 . H_2O$
Zn	0.2	Zinc chloride	$ZnCl_2$
Mo	0.01	Ammonium molybdate	$(NH_4)_6Mo_7O_{24} . 4H_2O$
B	Variable	Boric acid	H_3BO_3

The first crop was cut on February 26 and discarded for the purpose of purifying the sand and of obtaining larger plants. The second crop, on which all experimental data were recorded, was harvested when plants receiving 1.0 and 2.0 p.p.m. of boron were in the early bloom stage, i.e., on April 4. The fresh weight was taken, and a sample was oven dried for chemical analysis.

Histological studies were made on fresh material, using free-hand sections stained with one per cent neutral red. Some of this material was killed and stored in formalin-acetic-alcohol (F.A.A.), embedded in paraffin, sectioned 10 μ thick, and stained with safranin and fast green for more detailed studies and photomicrography. Stem sections were cut at approximately one centimetre from the growing point. Specimens collected during field surveys were stored in F.A.A. as soon as collected and studied later by the same technique.

HISTOLOGY OF THE NORMAL ALFALFA STEM

Young alfalfa stems are somewhat rectangular in cross-section, with four main vascular bundles, one at each corner. Several smaller bundles are situated between the main bundles. Vascular bundles are disposed between the pith and the cortex and separated by medullary rays. A group of collenchyma cells opposite each corner vascular bundle forms a strand of mechanical tissue (Figure 1).

The vascular bundles are all collateral. Xylem elements, vessels, fibres, and parenchyma, are thick-walled except for one or two rows near the cambium (Figure 2) which are thin-walled and are not lignified. The presence of such large thin-walled xylem vessels near the cambium is an important characteristic of healthy tissue. The phloem is limited by a strand of pericyclic fibres which forms a sort of bundle cap and abuts the endodermis.

The vascular tissues of the midrib of the leaf are similar to those found in the stems. The mesophyll consists of two rows of palisade cells and of loosely arranged spongy parenchyma.

HISTOLOGY OF ALFALFA STEM FROM BORON-DEFICIENT PLANTS

The most characteristic histological abnormalities caused by a deficiency of boron appear in the vascular bundles. Tissues most affected are the cambium and phloem. The first noticeable derangement is the cessation of normal differentiation of cells from cambium into xylem and phloem elements, followed by the disappearance of a normal cambium. This condition results in the absence of large thin-walled unlignified xylem vessels in the stems of affected plants. This feature permits the diagnosis of doubtful specimens which are on the border line and on which the visual symptoms are very faint or even absent. Cessation of cell differentiation, however, is not associated with cessation of cell division. On the contrary, cell division seems to go on and is apparently stimulated, resulting in hyperplasia of the cambium and phloem tissues. This hyperplasia brings about a wider separation of the xylem from the normal phloem and bundle cap. Hyperplasia in the cambium-phloem region is soon followed by cell elongation in the radial direction (Figure 3). Instead of having their long axis longitudinally with the stem, cells appear to elongate radially. This

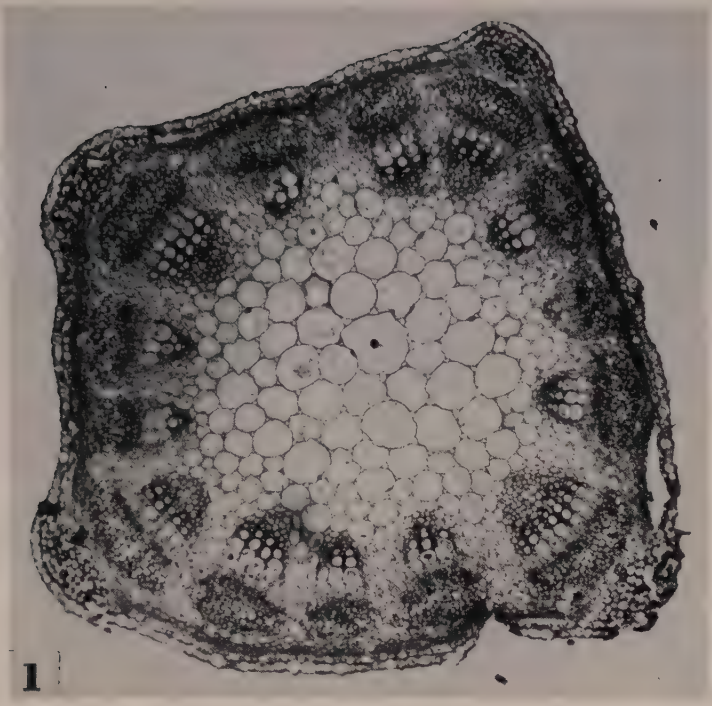

FIGURE 1. Photomicrograph of cross-section of stem of healthy alfalfa showing arrangement of vascular bundles as well as collenchyma bundles opposite each corner bundle. (×75).

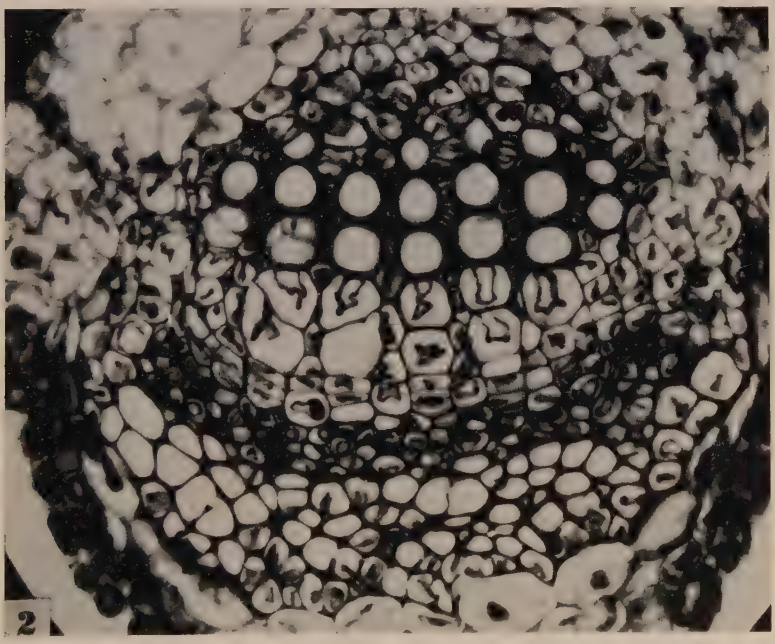

FIGURE 2. Photomicrograph of cross-section of healthy vascular bundle. The xylem vessels near the cambium are not lignified. The phloem is made up of four to five rows of cells and the bundle cap of as many large sones. (×400).

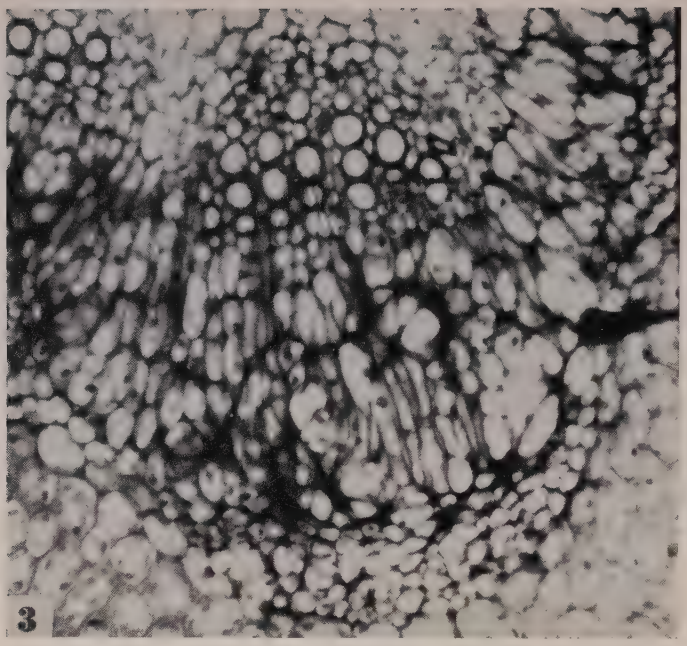

FIGURE 3. Photomicrograph of cross-section of vascular bundle from boron-deficient alfalfa plant. Note absence of thin-walled xylem vessels and cambium and wide separation of the xylem from the normal phloem and bundle cap. The tissue between the xylem and phloem shows hyperplasia, radial cell elongation, and some hypertrophy. (×360).

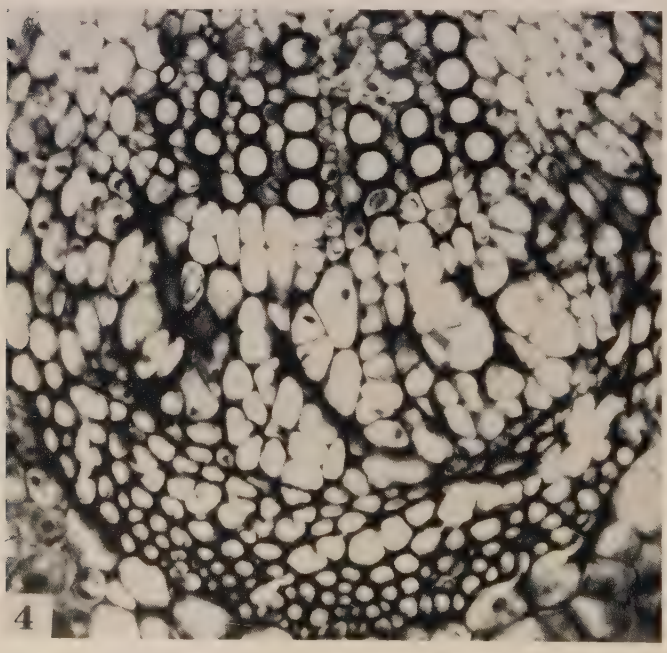

FIGURE 4. Photomicrograph of cross-section of vascular bundle of diseased alfalfa stem showing absence of normal cambium and thin-walled xylem vessels, hypertrophy of cells in the cambium-phloem region, cell crushing, and disintegration. (×360).

becomes quite evident on studying a longitudinal-tangential section cut in a vascular bundle between the xylem and phloem. Eventually, the radially elongated cells become hypertrophied and enlarge in any direction (Figure 4). This results in a still wider separation of the xylem from the bundle cap and in the crushing of normal phloem. Some of these hypertrophied cells are also crushed and finally disintegrated, forming masses of amorphous material which takes a dark stain with a safranin-fast green combination (Figure 4). In extreme cases, at the very tip of a stem or in small leaf veins, all tissues that occupy the space of normal cambium and phloem are disintegrated and stain darkly.

All stages described above can occur in the same stem at the same level in different bundles. These different stages can also be found in the same stem but at different levels. Through a study of serial sections of a stem, it was possible to follow the sequence of events in the establishment of histological boron-deficiency symptoms. The first symptoms appear in the most physiologically active tissues, i.e., the apical meristems, and progressively develop down the stem; by the time the growing point is dead, the deficiency may just be beginning to show at the base of the stem.

Xylem elements, as a rule, are little disturbed. However, vessels are not infrequently occluded and sometimes malformed as a result of hypertrophy of xylem parenchyma.

In the pith, it is not uncommon that some cells become hypertrophied and crushed against the vascular bundles, thus forming a ring of disintegrated tissues (Figure 5). Slight hypertrophy may also occur in the chlorenchyma of the cortex, the cells becoming elongated in the radial direction. Collenchyma bundles opposite each corner vascular bundle are usually crushed and form a mass of disintegrated tissues which stain darkly.

The vascular bundles of midribs and largest veins usually show disturbances similar to those in the stem. The mesophyll, however, presents an interesting picture. Both the palisade tissue and the spongy parenchyma become so closely packed that there are no air spaces left, resulting in increased thickness, rigidity, and crispness of the leaves.

THE RELATION OF HISTOLOGICAL ABNORMALITIES TO BORON SUPPLY

Results obtained indicate that severity of histological symptoms due to boron deficiency appears to be correlated with the amount of boron in the nutrient solution and the concentration of active boron in the tissues. The most severe symptoms of boron deficiency, hypertrophy and cell disintegration, were found in all plants receiving 0.0, 0.01, 0.10 p.p.m. of boron. Treatments of 0.25 p.p.m. produced milder symptoms characterized mainly by hyperplasia and radial cell elongation. Some of the stems, however, looked perfectly normal except for the absence of thin-walled xylem vessels. Plants receiving 0.50 p.p.m. looked perfectly healthy; however, histological examination of the stems revealed that they had received subminimal quantities of boron. The absence of young unlignified xylem vessels was a general feature in these stems. Chemical

analysis of plant tissue showed that the active boron content increased progressively with increased concentration of boron in the nutrient solution, whereas the severity of histological boron-deficiency symptoms decreased. Plants receiving 0.75, 1.0 and 2.0 p.p.m. of boron were apparently normal.

All the abnormalities described above for greenhouse material were also observed under field conditions, and ranged from severe hyperplasia and cellular disintegration to slight hyperplasia with absence of thin-walled, unlignified xylem vessels. This observation suggests a similar cause of the abnormalities under both sets of conditions.

DISCUSSION

The absence or subminimal supply of boron in the nutrient solution as well as in the soil causes definite and specific histological disturbances in alfalfa which are analogous to those observed on several other species of plants (1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15). The severity of these disturbances is dependent upon two factors: the actual boron concentration in the nutrient solution and the age of the plant. Symptoms of deficiency become evident sooner in plants receiving very minute amounts of boron, i.e., 0.10 p.p.m., than in plants receiving more, i.e., 0.25 p.p.m. Nevertheless, if plants grow long enough, symptoms are just as severe in the latter case as they are in the former one. Time factor compensates for a higher concentration of boron and the plant reaches a greater development in size before histological disturbances are comparable to those observed a few weeks earlier in a specimen which received smaller amounts of boron.

Abnormalities appear first in the apical meristems and near the growing points. Consequently, sections should be cut one or two cm. from the tip either in the stem or in the leaf blade for proper examination and diagnosis. Nevertheless, in dealing with doubtful cases, it may be found advantageous to examine sections cut even closer to the growing points.

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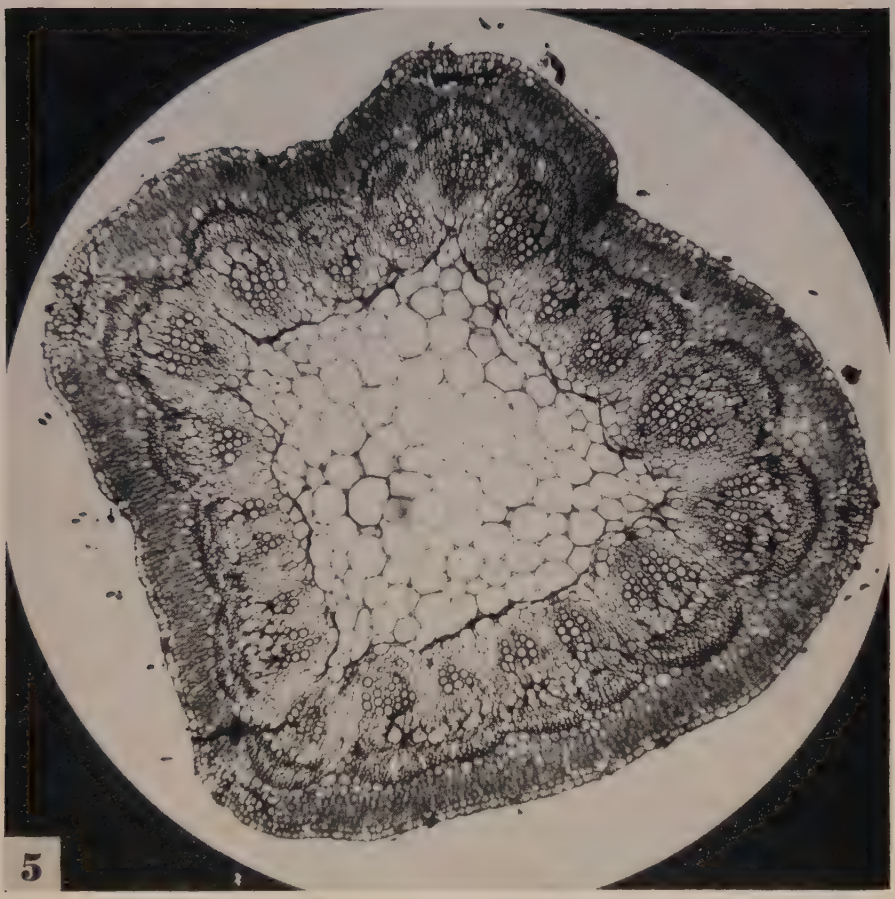

FIGURE 5. Photomicrograph of cross-section of stem of boron-deficient alfalfa plant showing hypertrophy and formation of dark masses of disintegrated tissue in the cambium-phloem region, cell crushing, and disintegration in the pith adjacent to the vascular bundles and collapse of the collenchyma bundles. (×60).

SOIL AND PLANT ANALYSIS AS MEANS OF DIAGNOSING BORON DEFICIENCY IN ALFALFA IN QUEBEC[1]

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[Received for publication February 18, 1954]

ABSTRACT

Boron deficiency was found in 119 of the 266 alfalfa fields surveyed in Quebec from 1950 to 1953, inclusive. Of that number, 61 per cent were located on light-textured soils, 45 per cent on medium and 14 per cent on heavy. The critical level of boron in alfalfa, below which the foliage was abnormal, was 15 parts per million. Plant tissue analysis afforded a more reliable criterion than soil analysis for the purpose of determining whether or not alfalfa was adequately supplied with boron. However, if provision is made for more boron in heavy than in sandy soils in order to avoid a deficiency of that element, soil analysis becomes a rather dependable indication of the boron status of a field. This work indicates that the heavy soils of Quebec should contain approximately 0.8 pound per acre of water-soluble boron and the sandy soils approximately 0.5 in order to support normal alfalfa. The data obtained by analysing alfalfa plants grown in sand cultures containing various concentrations of boron and those obtained by analysing the samples collected in the field, as related to the occurrence of deficiency symptoms, are in good agreement.

INTRODUCTION

The increasing need of boron fertilization for the production of alfalfa hay and seed in Quebec, as well as elsewhere, makes it desirable to further study methods of diagnosing boron deficiency. Thus, the purpose of the present paper is to correlate the contents of boron found in soil and in plant tissue with the visual and histological symptoms of a deficiency of this element in alfalfa.

According to Naftel (11), the addition of 0.3 part per million of boron in the form of borax to soils at various levels of liming was quite effective in the development of crimson clover heads and total plant yields. Woodbridge (17) concluded, from a large number of analyses, that soil concentrations of water-soluble boron above 0.5 part per million, on the dry weight basis, were associated with a general freedom from drought spot and corky core of apple. Estimates of the level of water-soluble boron in the soil that is critical for the alfalfa crop have ranged from 0.35 part per million, as reported by Reeve and others (13) for New Jersey, and Dawson and Gustafson (4) for the state of New York, to 1.0 part per million, as suggested by Dregne and Powers (6), for the soils of Oregon. Boron deficiencies were apparently present in alfalfa when grown on heavier soils of Illinois which contained less than 0.5 part per million of water-soluble boron. The critical level for sandy soils in that state was about 0.30 part per million (15).

As reported by Rogers (14), alfalfa grown on untreated soils contained less than 6.0 parts per million of boron in almost every case where response to borax was obtained. However, it contained more than 10 parts per million in 82 per cent of the cases where borax had been applied. The

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critical values reported in the literature for the boron content of alfalfa plants vary from 6.9 parts per million as given by McLarty and associates (9), to 23 parts per million as found by Munsell and Brown (10). Within this range, 9.0 parts per million were suggested by Dible and Berger (5), 10 parts per million by Powers (12), 15 parts per million by Dunklee and Midgley (7), and, finally, 20 parts per million by Dawson and Gustafson (4) and Bear and Wallace (1). On the other hand, Dregne and Powers (6) found some normal alfalfa plants in which the boron content varied from 12 to 22.5 parts per million. Whetstone and his co-workers (16) obtained a response to boron on Cecil clay with plants containing from 13 to 17 parts per million of that element, whereas no response was obtained on Huntington silt loam with plants containing from 12 to 19 parts per million. Most of the above figures for the boron content of alfalfa apply to the entire aerial portion of the plant, except those given by Munsell and Brown and by Dible and Berger which were obtained by analysing the leaves and the apical portion respectively.

From the literature cited above, it appears that a response to borax by alfalfa is likely to occur in cases where the soil contains less than 0.30 part per million of water-soluble boron, and the plant less than 10 parts per million of total boron. However, it should be emphasized that great variations were found, presumably due to variations in such factors as exchange capacity, exchangeable calcium content, and organic matter percentage of the soil. In view of the variations in the results that have been recorded, the authors undertook a similar investigation and the results are described in this paper.

MATERIALS AND METHODS

Survey

Alfalfa plants with corresponding soil samples were collected from 266 fields in various parts of the province of Quebec in the summers of 1950, 1951, 1952 and 1953. In order to minimize the effects of climatic conditions upon the boron content of the plants and the expression of deficiency symptoms, an attempt was made to conduct that survey within as short a period as possible every year. Consequently, approximately 80 per cent of the samples were collected from the middle of August to the middle of September, although the entire collection period for the four years ranged from the end of July to the end of September. Alfalfa was the dominant species in all fields; however, in many fields some other species such as timothy and red clover were also present. Each plant sample represented a composite of several plants cut just above the ground from various locations in the field. Borings to a depth of approximately 8 inches were taken at the point of plant sampling, and composited to obtain a soil sample to correspond with each plant sample. At the time of each sampling, observations of any visible physiological disorders were made, and notes on the general appearance of the crop were taken. In some cases, an appropriate sample was brought to the laboratory and submitted to microscopic studies, in order to detect any histological disturbances that were present.

Water-soluble (available) boron in soil was determined by extraction with hot water and subsequent application of the quinalizarin colour reaction, in accordance with the method of Berger and Truog (2). Total boron in plant tissue was also determined by means of the quinalizarin method, following the ashing of a 0.50-gram sample of plant material in a muffle furnace at 450° C. for 8 hours. The hydrometer method, as proposed by Bouyoucos (3), was used to determine soil texture.

The most specific visual symptom of boron deficiency in alfalfa is a stunting of the growing part of the plant. The consequent shortening of the main stem brings the upper branches close together with the formation of a so-called rosette. This disorder is always accompanied to a variable degree by a yellowing of the upper leaves, while the lower leaves remain green. Occasionally reddening was observed instead of yellowing. In a severe deficiency, the flower buds do not develop at all; if the deficiency is less severe, the buds will open, but die soon after. The histological features of the alfalfa affected with yellowing and taken from the fields surveyed were comparable to those already described for material grown in quartz sand (8). All degrees of abnormalities, from the absence of thin-walled xylem vessels and slight hyperplasia to severe hypertrophy and cell disintegration in the cambium-phloem region, were observed.

Greenhouse Studies

In order to compare the results of the survey with those obtained under controlled conditions, a sand culture investigation with alfalfa was begun in the fall of 1951. Seeds of alfalfa were sprouted in quartz sand. When the seedlings were approximately 2 inches in height, they were transferred to 2-gallon jars provided with one hole at the bottom and filled with quartz sand. Three seedlings were placed in each jar. To a basic nutrient solution adapted to alfalfa, i.e. high in calcium and potassium, the following concentrations of boron in parts per million were added: 0.0, 0.01, 0.10, 0.25, 0.50, 0.75, 1.0 and 2.0. These treatments were replicated five times. Five hundred millilitres of the appropriate nutrient solution, having a pH value of approximately 5.2, was added to each jar once a week. In order to reduce the accumulation of boron in the substrate, all jars were washed with one litre of distilled water 24 hours before adding any new solution. Water losses by evaporation and transpiration were made up regularly according to needs. All plants were cut when the best treatments reached the early bloom stage. The material from the first cutting was discarded as a means of eliminating any effect that may have resulted from possible traces of boron in the quartz sand.

RESULTS AND DISCUSSION

Relation between Visual and Histological Symptoms

In order to verify the reliability of the visual symptoms of boron deficiency observed in the field, a certain number of samples were submitted to microscopic study. The number of such samples amounted to 72 in 1950, 50 in 1951, and 21 in 1952. The results thus obtained are summarized in Table 1. Among the 63 samples taken from plants which exhibited visual symptoms of boron deficiency, only 3 were found completely free

TABLE 1.—RELATION BETWEEN VISUAL AND HISTOLOGICAL SYMPTOMS OF BORON DEFICIENCY OBSERVED ON 143 SAMPLES OF ALFALFA DURING THE YEARS 1950, 1951 AND 1952

Year	Samples free from visual symptoms, and on which histological symptoms were:		Samples with visual symptoms, and on which histological symptoms were:				Total number of samples submitted to microscopic studies
	Absent	Present	From very to rather pronounced	Very faint	Doubtful	Absent	
1950	47	1	21	1	0	2	72
1951	25	0	21	1	2	1	50
1952	7	0	10	2	2	0	21

from histological symptoms, while 5 were classified as doubtful. Moreover, histological disturbances were observed on only one of the 80 fields which were believed to be free from visual symptoms of that deficiency.

Therefore, the visual and histological examinations have disagreed completely in only 4 of the 143 cases included in that study, and they have agreed in 94 per cent of the cases. Of the 64 samples assessed as being boron-deficient on the basis of visual symptoms, 11 per cent showed no obvious histological symptom of deficiency. However, the visual symptoms were very faint on 5 or 6 of those samples. The histological disorders observed were quite characteristic of boron deficiency and could not very easily be mistaken for any other known nutritional symptoms. Consequently, visual observations made by an experienced worker seem to constitute a reliable means of determining whether or not boron is lacking in alfalfa. For that reason, and also because microscopic studies have not been made for all 266 fields surveyed, visual symptoms alone will be mentioned in the subsequent parts of this paper.

Plant vs. Soil Analysis

The frequency distributions of the boron deficiency symptoms observed on the field as related to the boron content in plant tissue and soil are given in Tables 2 and 3, respectively. The results indicate that visual symptoms of boron deficiency have been noted on 119 or 45 per cent of all fields surveyed. This does not actually mean that 45 per cent of the alfalfa growing in Quebec is boron-deficient, because only a small percentage of the plants in those 119 fields exhibited such symptoms. As a general rule, from 5 to 15 per cent of the plants were affected. In a very limited number of fields, however, the percentage of affected plants was as high as 50 per cent. In Table 2, there seems to be a sharp dividing line between the 14 and 15 parts per million levels of total boron in plant tissue. Not one sample containing more than 14 parts per million exhibited any visible symptom of boron deficiency, and only 7 of the 126 containing less than 14 parts per million were free from such abnormalities.

On the contrary, no such dividing line is evident in Table 3. All levels of water-soluble boron in soil falling between 0.4 and 0.8 pound

TABLE 2.—FREQUENCY DISTRIBUTION OF THE OCCURRENCE OF BORON DEFICIENCY SYMPTOMS OBSERVED ON 266 ALFALFA FIELDS DURING THE YEARS 1950, 1951, 1952 AND 1953, AS RELATED TO BORON CONTENT IN PLANT TISSUE

Boron in plants, p.p.m.	Visual symptoms	
	Present	Absent
Below 8	40	0
8	11	0
9	14	1
10	19	1
11	7	0
12	14	2
13	7	2
14	7	1
15	0	9
16	0	9
Above 16	0	122

TABLE 3.—FREQUENCY DISTRIBUTION OF THE OCCURRENCE OF BORON DEFICIENCY SYMPTOMS OBSERVED ON 266 ALFALFA FIELDS DURING THE YEARS 1950, 1951, 1952 AND 1953, AS RELATED TO THE AMOUNT OF WATER-SOLUBLE BORON IN SOIL

Water-soluble boron in soil (lb./acre)	Visual symptoms	
	Present	Absent
Below 0.3	35	0
0.3	14	1
0.4	24	4
0.5	15	13
0.6	15	5
0.7	10	6
0.8	6	12
0.9	0	7
Above 0.9	0	99

per acre inclusively are associated with cases where the plant exhibited symptoms of boron deficiency and cases where it was entirely free from such disorders. To be more specific, it should be added that, among the 110 fields falling within the range above indicated, 40 were free from abnormalities and 70 had visual symptoms. In the present study, therefore, analysis of plant tissue afforded a more reliable criterion than did soil analysis for the purpose of deciding whether or not alfalfa was adequately supplied with boron.

Soil Texture as a Factor of Variation in Predicting Critical Levels of Water-Soluble Boron in Quebec Soils

Stinson (15) has reported that boron deficiency is likely to occur in alfalfa on the sandy soils of Illinois which contain less than 0.3 part per million of water-soluble boron, and on the heavy soils with less than 0.5 part per million. This relation between soil texture and the critical level of boron in the soil for alfalfa, if found in the soils of Quebec, may explain

the lack of relationship between the factors given in Table 3, i.e. water-soluble boron in soil and occurrence of visual symptoms of a deficiency of the element in the plant, since the soils which were sampled in this study varied widely in texture.

The results presented in Table 4 indicate that the observation made by Stinson in Illinois, as to the variation between light and heavy-textured soils, in so far as their critical level of boron is concerned, also applies to the soils of Quebec. For the purpose of the present study, the sand, gravelly loam and sandy loam textures have been arbitrarily classified as light; the loam, silt loam, sandy clay loam and silty clay loam as medium; and finally the clay loam and clay as heavy.

Table 4 shows that the alfalfa growing on 61 per cent of the light and 45 per cent of the medium-textured soils exhibited boron deficiency symptoms, whereas only 14 per cent of the fields on heavy soils were abnormal. The average water-soluble boron content of the light-textured soils associated with deficiency sumptoms was found to be 0.34 pound per acre, that of the medium-textured soils 0.48, and finally, that of the heavy-textured soils 0.60. However, in the class of soils producing normal alfalfa, the light-textured ones contained the most boron (Figure 1).

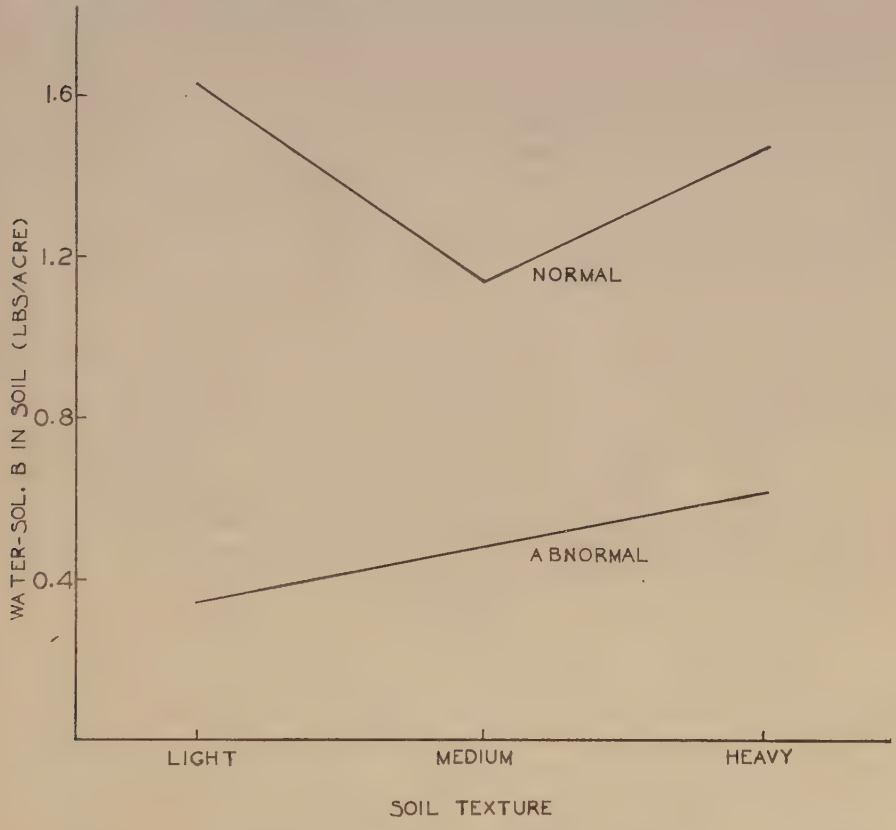

FIGURE 1. Water-soluble boron content of the soils producing normal and abnormal alfalfa as related to texture.

TABLE 4.—OCCURRENCE OF BORON DEFICIENCY SYMPTOMS ON ALFALFA AND WATER-SOLUBLE BORON CONTENT OF THE SOIL IN RELATION TO THE TEXTURE OF 261 FIELDS SURVEYED DURING THE YEARS 1950, 1951, 1952 AND 1953*

Soil texture	Total number of samples	Samples associated with symptoms		Water-soluble boron in soil (average)			
				Symptoms present		Symptoms absent	
		Number	Per cent of total	Lb./acre	Range	Lb./acre	Range
Light	138	84	61	0.34	0.0–0.6	1.63	0.3–4.8
Medium	58	26	45	0.48	0.1–0.8	1.14	0.5–3.1
Heavy	65	9	14	0.60	0.3–0.8	1.49	0.7–5.3

* Five samples of peat have been excluded from that study, because they were unusually high in water-soluble boron.

The sandy soils of Quebec are likely to lack boron for the normal growth of alfalfa if they contain less than 0.5 pound per acre of water-soluble boron. In the case of medium and heavy-textured soils, the critical levels seem to be 0.6 and 0.8 pound per acre respectively. Such a differential behaviour of various soil textures seems to account for the lack of relationship observed between boron content in the soil and the occurrence of boron deficiency sumptoms. Thus, the analysis of the soil appears to be as good a criterion as the analysis of the alfalfa plant to determine the boron status of a field, provided that the worker knows the texture of the soil, and takes into account the fact that heavy soils must contain more boron than sandy soils in order to avoid deficiency trouble.

Relation between Total Boron in Plant and Water-Soluble Boron in Soil

Figure 2 shows the relationship between the amount of boron in the aerial portion of the alfalfa plant, and the quantities of that element in the soil where the plants are grown. An increase in the amount of boron in the soil up to approximately 2.5 pounds per acre appears to result in a similar increase in the content of this element in the plant; above that point, the boron content of the plant has a tendency to level off, which seems to indicate that boron is not likely to become toxic to alfalfa under normal field conditions. Since alfalfa continues to take up appreciable amounts of boron, even after it has reached the point where deficiency symptoms are not likely to appear, it is probable that far greater quantities of boron are needed to produce maximum yields of forage and seed, than are required to avoid deficiency symptoms on the foliage.

Greenhouse Results

The information obtained from the greenhouse test is summarized in Table 5. The analysis of the sand for hot water-soluble and total boron, at the end of the experiment, indicates that difficulties were encountered in keeping the intended levels of boron in solution. There is also a good indication of some boron being fixed in a form unavailable or difficulty available to plants. Moreover, it is very likely that the feeding power of plants growing in soil and that of plants growing in artificial sand cultures

TABLE 5.—YIELD, BORON CONTENT OF PLANT TISSUE AND SYMPTOMS OF BORON DEFICIENCY AS RELATED TO THE CONCENTRATION OF HOT WATER-SOLUBLE AND TOTAL BORON IN SAND CULTURES (AVERAGE OF 5 REPLICATIONS AT THE SECOND CUTTING)

B in nutrient solution, p.p.m.	B in sand (3 samplings for each replicate)*		Yield in grams/jar (green wt.)	B in plant p.p.m.	Deficiency symptoms	
	Water-soluble, p.p.m.	Total, p.p.m.			Histological abnormalities in vascular bundles	Visual
0.00**	None	0.04	23.3	4.0	Severe hypertrophy and cell disintegration	General yellowing—Necrosis. Some dead leaves—Plants stunted.
0.01	None	0.05	29.1	4.4	Severe hypertrophy and cell disintegration	General yellowing—Necrosis. Plants stunted.
0.10	0.08	0.26	47.6	9.4	Hyperplasia and severe hypertrophy	Yellowing of young leaves—Rosette.
0.25	0.14	0.82	56.8	12.8	Hyperplasia and hypertrophy	Death of reproductive buds.
0.50	0.15	1.99	74.1	15.4	Absence of unlignified xylem vessels	Normal.
0.75	0.30	3.30	73.7	16.6	Normal	Normal.
1.0	0.67	3.06	80.5	31.2	Normal	Normal.
2.0	1.81	6.14	72.2	49.0	Normal	Normal.
L.S.D. at 1% level	0.08	0.43	7.1	2.9		

* Samples taken at the time of harvesting; the nutrient solution had been added 3 days prior to that time.
** Under the conditions of the experiment, it was found impossible to obtain a substrate completely free from boron.

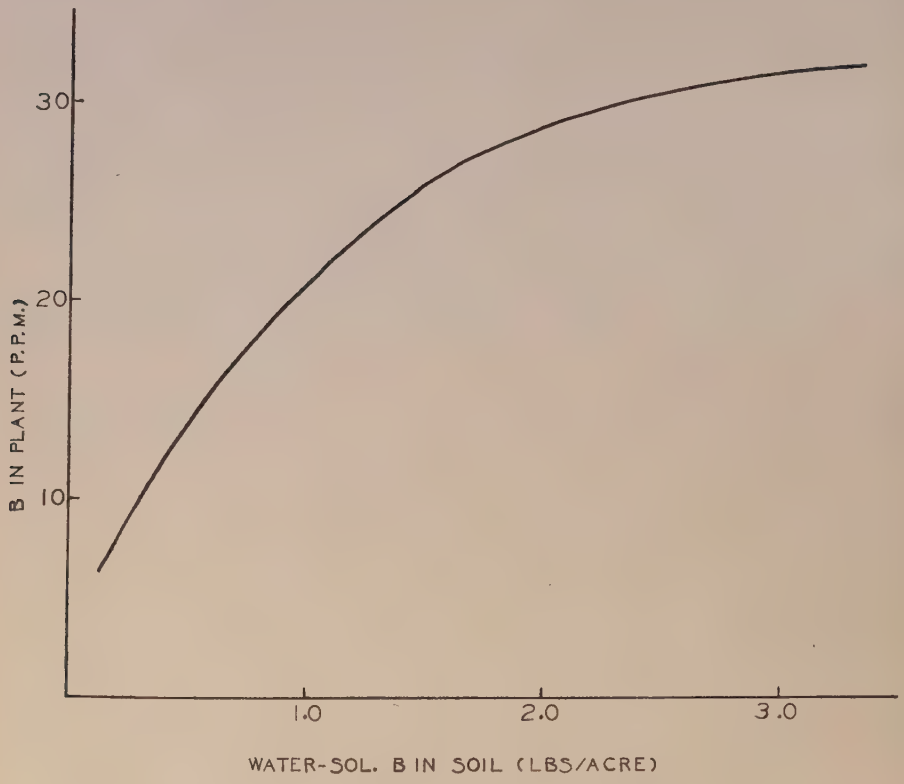

FIGURE 2. Boron in alfalfa as related to the amount of water-soluble boron in soil.

are not comparable; therefore, no attempt will be made to correlate boron concentrations in sand cultures and the yields of alfalfa thus obtained. However, the data obtained in the greenhouse, as to the correlation between the boron content of the plant and the kind as well as the amount of growth, substantiate those obtained from the above reported survey. Steady and significant increases in the yield of plants growing in the greenhouse corresponded to significant increases in the boron content of the plant up to approximately 15 parts per million (Figure 3). Similarly, neither external nor internal deficiency symptoms were observed above that point. A significant decrease in yield was experienced with the plants receiving the nutrient solution containing 2.0 parts per million of boron, although they did not exhibit any toxicity symptoms. Such a situation could be due to either the direct action of boron or the counteraction of that element upon the uptake or assimilation of another element.

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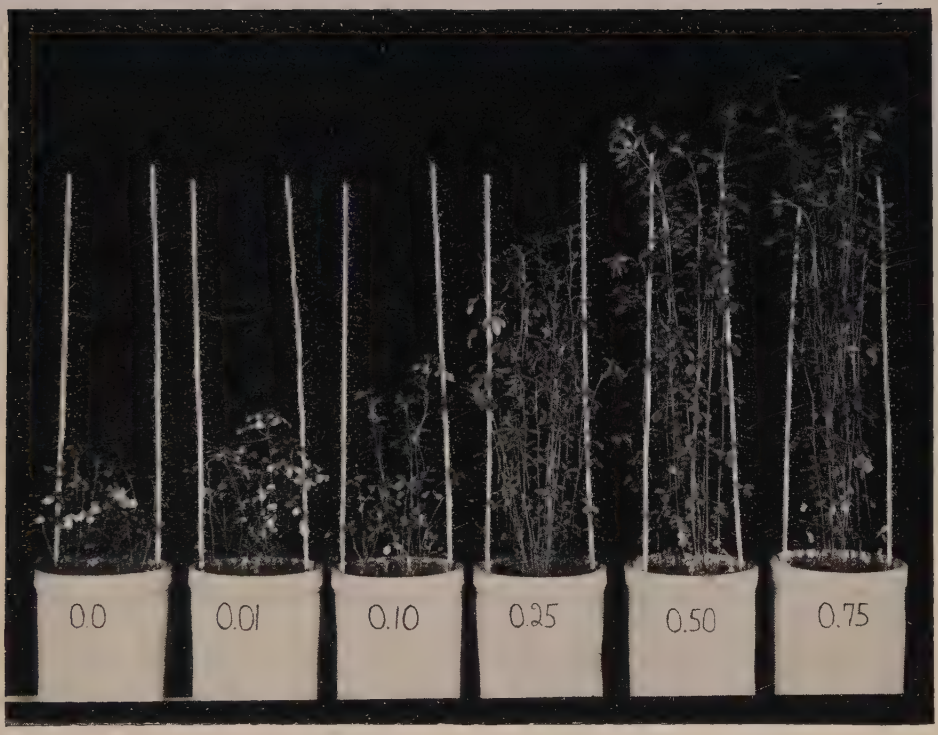

FIGURE 3. View of some of the material growing in the greenhouse; figures on the jars indicate boron concentrations in parts per million of the nutrient solutions.

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PINK ROT OF POTATO TUBERS ON VANCOUVER ISLAND[1]

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[Received for publication February 2, 1954]

ABSTRACT

The pink rot disease of potato, caused by the fungus *Phytophthora erythroseptica* Pethybr., was found in a garden near Victoria, British Columbia, during August, 1953. This disease has not been recorded from the coastal areas of the Province. In pathogenicity tests, the fungus proved to be pathogenic to potato tubers and to seedlings of tomato and spinach, but seedlings of radish, turnip, cabbage, pea, garden beet, and corn were not attacked. Sporangia were produced on infected seedlings in moist soil and in water, and also on diseased potato-tuber tissue in water. Control of the disease involves crop rotation, adequate soil drainage, sound irrigation practices, and the disposal of diseased plants and tubers.

INTRODUCTION

During August 1953, approximately 20 per cent of the plants of the Warba variety grown in a garden near Victoria, British Columbia, were found to have died prematurely. A few of these exhibited 'axillary tuber' symptoms similar to those usually attributed to infection of the basal parts of the stem of potato plants by the fungus *Rhizoctonia solani* Kühn.

When the tubers were dug, however, many of them were partially or entirely rotted, and a considerable amount of soil was found clinging to the badly rotted tubers, due to the growth of mycelium from the tissues into the surrounding soil.

When the diseased tubers were cut and further examined, the symptoms were found to be similar to those of pink rot caused by *Phytophthora erythroseptica* Pethybr. which was found in 1943 in the interior of British Columbia and described by Jones (3). This disease, however, has not been recorded from the coastal areas of British Columbia.

The main symptoms of the disease on the tubers were the dark-purple discoloration of the lenticels, the firm but watery texture of the tissues, and the change in colour of the flesh from dull white to salmon-pink after being cut and exposed to the air for 15 minutes. A sticky exudate was also often evident on the lenticels (Figure 1).

Tissue sections from diseased tubers were placed in water overnight at room temperature, and, in the morning, numerous sporangia, typical of *Phytophthora* spp., were evident (Figure 2). After a few days, numerous oogonia and amphigynous antheridia were also present, and, a few days later, many oospores had been produced. The approximate minimum and maximum diameters of the oospores are 23.0 and 36.5 μ, respectively with a mode of 29-32 μ. These dimensions and the amphigynous character of the antheridia agree with those given by Pethybridge (4) in his original description of *Phytophthora erythroseptica* from Ireland.

The fungus was isolated, cultured on agar media, and its pathogenicity to potato tubers of the Netted Gem and White Rose varieties established.

[1]Contribution No. 1365 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2]Associate Plant Pathologist.

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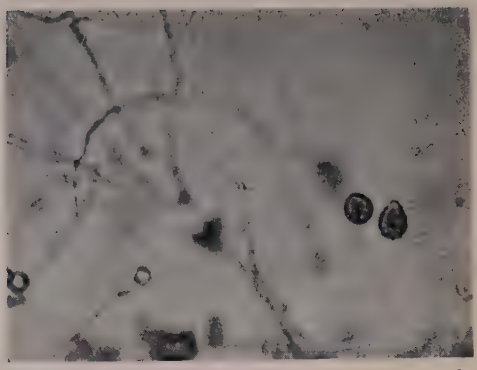

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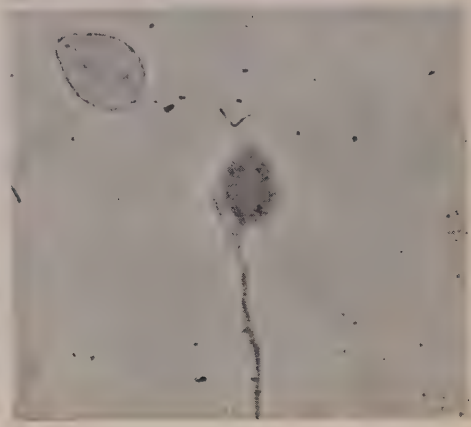

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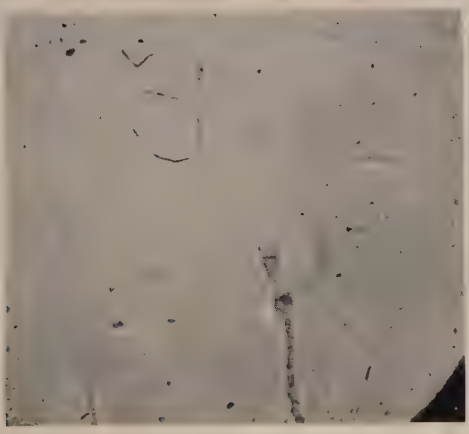

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FIGURE 1. Naturally infected potato tubers of the Warba variety. Note the exudate on the lenticels.

FIGURE 2. Sporangia of *P. erythroseptica* on diseased tuber tissue in water.

FIGURES 3, 4, and 5. Sporangia of *P. erythroseptica* on tomato seedling. FIGURE 4 shows two mature sporangia a few minutes before emergence of zoospores. FIGURE 5 the same sporangia as in Figure 4, a few minutes after emergence of zoospores. Note the septum and plug of upper empty sporangium.

FIGURE 6—Sporangia of *P. erythroseptica* on spinach seedling.

SUSCEPTIBILITY OF SEEDLINGS OF SEVERAL GARDEN CROPS TO INFECTION BY THE FUNGUS

With a soil-inhabiting fungus of this nature, one obvious method of control is to practise a good rotation. In gardens of limited acreage, this would prove rather difficult without some knowledge of the susceptibility of different garden crops to infection by the fungus. With that in mind, an experiment was carried out to test the susceptibility of the seedlings of cabbage, radish, turnip, corn, garden beet, pea, tomato and spinach.

In one test, the seeds of these plants were sown in the greenhouse in pots containing sterilized soil, using three pots for each kind of plant. When the seedlings were about 2 inches high, small slices from artificially inoculated potato tubers, which had been kept in water for a few days until sporangia had developed on them, were placed adjacent to the stems of the seedlings at soil level in one pot, and slightly below soil level in another one. One pot was set aside as a control, and the soil in all of them was kept moist.

Within approximately 10 days, symptoms of stunting and wilting were observed on several of the spinach and tomato seedlings, but such symptoms were not seen on the cabbage, turnip, radish, corn, pea and garden-beet seedlings. Infection by the fungus was considered positive only when, upon microscopic examination, sporangia of the fungus were visible on the roots and stems of the seedlings which had been placed in water in syracuse dishes for a few days (Figures 3, 4, 5, and 6). On some of the seedlings, it was observed that sporangia had developed on the roots and basal portions of the stems, before the young plants were lifted from the soil.

In another test, the different seeds were sown in deep flats containing sterilized soil mixed with pieces of artificially inoculated tubers. Another flat containing sterilized soil without inoculum was set aside as a control. No disease developed in the control flat but in the inoculated flat the spinach and tomato seedlings became infected, and, after placing in water, sporangia of the fungus developed from them. The other seedlings did not show symptoms of infection.

DISCUSSION AND CONCLUSIONS

In the pathogenicity experiments with the fungus, the varieties White Rose and Netted Gem were tested. Cairns and Muskett (2), reporting from Ireland, tested 35 varieties in the field and 30 varieties by tuber inoculation, and none showed any marked resistance. In British Columbia, Jones (3) inoculated tubers of the varieties Epicure, Warba, Irish Cobbler, Green Mountain, Netted Gem, Burbank, Columbia Russett and Sequoia, and all of them were susceptible. It is doubtful if any of the potato varieties are immune to attack by the fungus, although Tompkins and Tucker (5) reported that a strain of *P. erythroseptica*, isolated from diseased pink calla (*Zantedeschia rehmannii* Engler) was non-pathogenic to potato tubers of the White Rose variety.

A survey of the literature indicates that the host range of the fungus is limited, particularly on growing plants. Buddin (1) reported that *P. erythroseptica* caused a shoot rot and shanking of tulips in England, and that the same species, isolated from potato, was able to infect tulips. In the host-susceptibility test, the spinach and tomato seedlings were attacked

by the fungus, but the cruciferous and other seedlings were not affected. It thus appears that tomatoes and spinach should be avoided in garden rotation crops if the disease is known to have occurred, especially as the practice of crop rotation has been recommended widely as a control measure.

The garden where the disease was found is on high land, and the soil well drained, but there was a slight seepage from a neighbour's septic tank into one area, and the grower claimed that the disease was first observed in that area. It is thus probable that the fungus was present in the garden soil or in the seepage water, and, under these moist conditions, zoospores would probably emerge from the resting oospores, attack the roots, underground portions of the stems, stolons and tubers of the potato plants. Under moist conditions, the initial infection of a few plants would be followed by the infection of other plants and of tubers by the zoospores emerging from the sporangia, as it has been shown that sporangia develop readily on diseased tissues (Figures 2, 3, and 6). It is thus considered that good soil drainage is an essential factor in the control of the disease, and where overhead irrigation is practised, this should not be carried out too late in the season when the tubers are maturing, and when autumn rains are to be expected.

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APPARENT EFFECTS OF MANAGEMENT ON THE ORGANIC MATTER, NITROGEN AND PHOSPHORUS CONTENTS OF A BROWN FOREST SOIL[1]

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[Received for publication April 14, 1954]

ABSTRACT

Four areas of brown forest soil of the Grenville series were studied. These were located on the Macdonald College property near Ste. Anne de Bellevue, Que. Different management and cropping practices had been followed on these areas during the past 20 to 40 years, the extremes being continuous cultivation and continuous sod. Manuring and fertilization also differed. Analysis showed that organic matter, total nitrogen, organic phosphorus and cation exchange capacity increased, whereas Bray adsorbed phosphorus decreased, both significantly, as the intensity of cultivation decreased.

It is pointed out that available information strongly suggests that the cation exchange capacity of most soils of Eastern Canada is predominantly organic in nature.

The possibility of the acceleration of the accumulation of a store of available phosphorus in soils in organic form when these soils are under cultivation, including sod crops, is discussed.

Soils of the Grenville series occupy a considerable area in Eastern Ontario and Southwestern Quebec. They are till soils belonging to the brown forest group. Descriptions are given in various soil survey bulletins (7, 8). In the vicinity of Montreal, Que., these soils are used rather extensively for market-gardening. Under present conditions the maintenance of their organic matter content when used for this purpose constitutes an important problem. An investigation of the effects of management on the organic matter content and related characteristics of these soils, therefore, was desirable.

EXPERIMENTAL METHODS

In early summer 1952 areas representative of Grenville clay loam occurring on the Macdonald College property were selected for sampling. Three of these, A, B, and C, were on nearly flat topography; the fourth, the continuous sod area D, was on a gentle slope. It was considered probable that none of these areas had suffered a significant amount of erosion. Later inspection of the analytical data obtained, in respect of variability of organic matter content within the cultivated blocks A, B and C, showed no indication of erosional loss from or addition to these areas.

The principal differences in management represented at the sites chosen were the proportion of the time the land was under cultivation, and the kind and amount of fertilization received. These differences, together with statements relative to cropping practices followed, are summarized on next page:

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Macdonald College Journal Series No. 351.

[2] Postgraduate students in Agronomy.

[3] Associate Professor of Chemistry.

[4] Associate Professor of Agronomy.

Area A.—Continuous cultivation for about 40 years with relatively heavy applications of farm manure (estimated at about 10 t. per acre per year) and of chemical fertilizer (about 1000 lb. of 4-12-6 per acre per year in recent years); green manures also are used. Small fruits and vegetable crops are grown.

Area B.—Land under sod crops 5 years in 7 as a rule; farm manure the only fertilizer used, sometimes enriched with stable phosphate. Usually alfalfa persists, in variable amounts, throughout the sod part of the rotation. Corn and grain are the other crops. Essentially this regime has been followed for about 40 years.

Area C.—Land under sod crops 2 years in 6 as a rule, with three crops of cereal grain and one of corn grown for grain; chemical fertilizer used at the rate of approximately 1300 lb. of 4-12-6 per acre per 6-year rotation, applied to the corn and grain crops. During the past 13 years all crop residues have been left on or returned to the land, grain only being removed. In the 6 preceding years total crops were removed in an effort to reduce weediness. Previously this area was managed similarly to Area B.

Area D.—Land continuously under sod for more than 40 years; grazed lightly to moderately; no fertilizer other than that contributed by grazing livestock has been applied. The present sward consists predominantly of grasses, weeds, and herbs, with variable proportions of wild white clover and wild vetch.

These were not experimental areas in the usual sense of that term and the management practice followed on blocks A, B and C has varied somewhat from time to time as necessitated, e.g., by failure of a legume crop and necessity to control weeds.

The extent of each of these areas was approximately 27,000 square feet. Within each of them 45 soil cores (2″ × 6″) were taken at intervals of 25 feet following the pattern of a rectangular grid. These cores were analysed individually for organic matter, total nitrogen and so-called adsorbed phosphorus. In addition, composite samples were taken from each area. These consisted of about 25 lb. of soil taken as spade slices from the 0–6″ layer at eight (in the case of area C, nine) symmetrically placed locations within each area. These composites were analysed for total and inorganic cation exchange capacity, organic phosphorus, texture and pH value. The primary reason for taking these composite samples was that the analysis of the individual core samples for phosphorus (by L.B.M.) was in progress when it was desired (by B.H.Y.) to investigate cation exchange capacity and texture. It also was realized that, since the weights of the individual core samples had not been taken before sieving, it was impossible to make accurate composites from the screened material.

Organic matter was estimated by a modification of the Walkley-Black procedure (11). A micro-kjeldahl method was used for the estimation of total nitrogen, employing mercury as the catalyst and filtering the digest before distillation. Adsorbed phosphorus was extracted by the laboratory method of Bray and Kurtz (2), and the inorganic phosphorus content of the extract measured by the Truog-Meyer procedure (14) after addition of bisulphite. Organic phosphorus was extracted by the method of Pearson (10) and the organically-bound phosphate estimated from the difference, total minus inorganic phosphate, as measured by the Truog-Meyer procedure before and after oxidation with magnesium nitrate. Total and inorganic exchange capacities were determined on 5.0 gm. samples of 2-mm. air-dry soil by a modification of the ammonium acetate procedure (4), the inorganic capacity after ignition under the conditions proposed by Mitchell (9). Mechanical analyses were made by the pipette method, oxidizing

with H_2O_2, omitting the HCl treatment, and using Calgon as the dispersing agent. The pH values were measured electrometrically at a soil-water ratio of about 1 : 2.

EXPERIMENTAL RESULTS AND DISCUSSION

On the basis of the results of mechanical analysis of the composite samples the textural classes of the soils from areas A, B, C and D were: sandy clay loam to clay, sandy clay, sandy clay loam to clay and sandy clay loam to clay, respectively. The pH values for these samples were 7.4, 7.1, 7.0 and 6.6, in the same order as for soil class.

Table 1 presents a summary of the statistical analysis of the results obtained for the organic matter content of the individual samples, one determination per sample, with the conclusions drawn from this analysis.

The corresponding data for total nitrogen content are shown in Table 2.

TABLE 1.—ORGANIC MATTER CONTENT

Area	Mean values, per cent	Coefficient of variability	Differences, successive pairs	Rating
D	9.30	22.40	3.38	1
B	5.92	10.42	0.14	2
A	5.78	7.43	1.82	
C	3.96	6.57		3
	D.R.S. for P = 0.01 is		0.732	

TABLE 2.—TOTAL NITROGEN CONTENT

Area	Mean values, per cent	Coefficient of variability	Differences, successive pairs	Rating
D	0.408	24.29	0.110	1
B	0.298	12.18	0.016	2
A	0.282	7.76	0.072	
C	0.210	12.43		3
	D.R.S. for P = 0.01 is		0.0318	

It may be noted that a relatively large coefficient of variability was to be expected for area D, since there is an appreciable degree of micro-

relief at this site and the distribution of the species composing the vegetative cover also is variable, and since all areas were sampled according to a fixed pattern.

The results obtained for the organic matter and total nitrogen contents of the soil at the various sites suggest that the cultural regimes practised in areas A and B are equally effective in respect of maintenance of organic matter and of total nitrogen, and that the practice followed in area C has been less efficient for this purpose.

The carbon-nitrogen ratios, calculated from the mean values given in Tables 1 and 2, are 11.9, 11.9, 11.0 and 13.2 for areas A to D in that order.

Analysis of the composite samples in quadruplicate—that from area D in triplicate only—and statistical analysis of the results gave the cation exchange capacity picture presented in Table 3. The organic cation exchange capacity, that is, the difference in exchange capacity before and after ignition at about 400° C., is given as a percentage of the total exchange capacity as determined before ignition.

TABLE 3.—CATION EXCHANGE CAPACITY (E.C.) IN M.E./100 GM.

Area	Mean values, total E.C.	Differences, successive pairs	Rating	Organic E.C. per cent of total E.C.
D	24.9	.....	1	71
B	20.3	..... 4.6	2	69
A	19.4	..... 0.9		65
C	15.9	..... 3.5	3	65

D.R.S. (total E.C.) for P = 0.05 is 2.83

It is evident that the major part of the cation exchange capacity of these soils is due to organic matter. The results also show that the value for this soil characteristic can be decreased significantly by depletion of the organic matter content. Unpublished data*, obtained in this laboratory, indicate that the cation exchange capacity of many, probably most, of the soils of Quebec and of the Maritime Provinces is due mainly to the organic fraction. Thus, organic cation exchange values for loam soils from Southeastern Quebec, obtained by Menzies, accounted for as much as 85 to 90 per cent of the total cation exchange capacity.

The data obtained in the analysis (in duplicate) of the core samples for content of adsorbed phosphorus are summarized in Table 4.

* Menzies, R. G. M.Sc. thesis. 1944.

TABLE 4.—ADSORBED PHOSPHORUS

Area	Mean values, p.p.m. P	Coefficient of variability	Differences, successive pairs	Rating
A	114.6	13.9	.....	1
			90.8	
B	23.8	92.4	.....	2
			11.6	
C	12.2	32.2	.....	3
			7.5	
D	4.7	55.1	.....	4
	D.R.S. for P = 0.01 is		7.54	

It will be noted that, whereas the relatively heavy fertilization of area A resulted in high values for adsorbed phosphorus, the moderate fertilization of area C has not sufficed to maintain adsorbed phosphorus at as high a level in this area as has the management and cropping practice followed in area B.

Customarily it is assumed that the inorganic phosphate in the Bray extract represents the 'available' phosphorus in a soil. It might be expected that the reagent employed would release some organically-combined phosphate also. Work in progress indicates that this is true in case of soil from area D and that, in fact, some two to three times as much organic as inorganic phosphorus is being released. An earlier investigation (3) on the dilute acid-soluble phosphorus in the Greensboro loam soil (a podsol), using the Truog extraction reagent, showed that this reagent also released some organically-combined phosphorus. These observations, together with the recent findings of Fuller and Rogers (6) demonstrating the availability of the organic phosphorus of barley flour to grass, suggest that in the evaluation of the 'available' phosphorus of soils some weight should be given to the organic as well as to the inorganic phosphorus extracted. Fuller and Rogers also found that, for the nutrition of grass, barley residues from the vegetative parts of mature plants provided a less readily available source of phosphorus than did less mature tissues, even though the phosphorus in both was present largely in inorganic form. They also reported that addition of nitrogenous fertilizer improved the uptake by grass of phosphorus from such barley residues.

Comparison of the adsorbed phosphorus values given in Table 4 with those for organically-combined phosphorus in Table 5 shows that these forms of phosphorus varied inversely in case of the soil areas under investigation.

The results obtained in the analysis (in triplicate) of the composite samples for total organically-combined phosphorus are given in Table 5.

TABLE 5.—TOTAL ORGANIC PHOSPHORUS

Area	Mean values, p p.m. P	Coefficient of variability	Differences, successive pairs	Rating
D	415	16.5	 218	1
B	197	5.8	 47	2
C	150	20.0	 59	3
A	91	47.3	.....	4
D.R.S. for P = 0.05 is			91 p.p.m.	

The content of organic phosphorus, it is seen, varies inversely with the extent of cultivation. This result is in agreement with the early work of Schollenberger (12) on soils in Ohio. Such results show that organically-combined phosphorus is depleted under cultivation, and suggest that the process might be reversed in land under sod.

Many of the soils of Eastern Canada have high fixative capacities for soluble phosphates, and the availability to crops of such phosphates when mixed with these soils decreases rapidly. It would seem desirable, in case of soils of this nature, to utilize organic forms of phosphorus which, like the organic forms of nitrogen, might be made available throughout the growth period by microbial action. The writers suggest that this end probably could be best achieved by assisting sod crops to accumulate and store phosphorus in such forms, and by adjustment of soil properties so as to favour liberation of this phosphorus in inorganic form when the sod is ploughed down. In support of this suggestion it may be noted that unpublished data, obtained in a co-operative experiment with the Department of Nutrition, Macdonald College, showed that super-phosphate applied in the spring as a top-dressing to a meadow established on soil of high fixative capacity for phosphate (Greensboro loam) resulted in a significant increase in the phosphorus content of the hay (non-leguminous). The increase was mainly in inorganic phosphate and it occurred mainly in the stem tissue where it was, incidentally, relatively unavailable to sheep. Further, recent investigations with radio-phosphorus (5, 13) have demonstrated a relatively high efficiency of uptake of fertilizer phosphorus following surface application to land in sod crops under humid climatic conditions. Such results as these suggest that, in humid climates at least, phosphate fertilization of sod crops may prove to be an effective means of increasing the supply of phosphorus available to succeeding cultivated crops grown on soils of high phosphorus-fixing capacities. Considerable investigation will be required to establish the practicability of such a procedure. Thus, data on the phosphorus content of the stubble, aftermath, and root systems of sod crops seem to be very scanty, and the writers know of no data on the increase in phosphorus content of such tissues which may be obtainable by

top-dressing with phosphate. Undoubtedly, the per acre storage capacity of such crop residues for phosphorus will be small. For example, on the basis of an average value of 0.2 per cent phosphorus (P) for timothy hay given by Beeson (1), and of a dry weight of stubble, aftermath and roots amounting to 4000 lb. per acre, the quantity of phosphorus stored would be only eight pounds per acre. Nevertheless, considerable amounts of organically-combined phosphorus do accumulate in land under sod. Possibly microbial action is a major factor in the conversion of inorganic to organic phosphorus in grassland soils. It is suggested that possibly such conversion may be promoted by appropriate fertilization of the sod crop.

ACKNOWLEDGMENT

The authors acknowledge with appreciation the receipt of financial assistance in support of this investigation from the Quebec Department of Agriculture.

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THE NATURE AND INHERITANCE OF SEED-COAT SPOTTING IN PERMEABLE-SEEDED STRAINS OF SWEET CLOVER (*Melilotus alba* L.)[1]

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[Received for publication March 8, 1954]

ABSTRACT

In line with previous work it was shown that permeability of the seed in certain lines of sweet clover depended on the occurrence of brownish, sunken spots on the seed coat. A genetic study showed that spotting was controlled by one major recessive gene but influenced by one or more modifying genes. Environment had a marked effect by reducing the percentage of spotted seed and the size of the spot on seeds produced under cotton selfing bags. Permeability was found to be dependent on the presence of the spot but was independent of the size of the spot. The controlling environmental factor affecting spotting was not determined but the expression of spotting appears to be greatest under good growing conditions.

A graftage experiment determined that the spot was not a lesion caused by a disease that could be transmitted systemically to non-spotted material. It was also shown that the spotted character was not transmitted directly by the pollen. Histological observations of the spot tissue showed the Malpighian cells to be shorter than normal, distorted and the lumen filled with a brown pigment. The osteosclerid cells appeared collapsed or crushed and the nutrient layer absent, while only the aleurone layer of the endosperm remained at the spot. Cotyledons were shown to be pressed against the seed coat at one or more places in the spotted area. These observations suggested three possible causes of the spottings: (*a*) The embryo may be feeding on the seed coat tissue or nutrient intended for the seed coat causing a distortion of the cells of the seed coat; (*b*) The cotyledons may be growing too large for the seed coat in certain areas thus crushing and distorting seed coat cells at those points; (*c*) Certain portions of the seed coat may be smaller than normal in relation to cotyledon size.

INTRODUCTION

During the last 40 years sweet clover has become one of Western Canada's most important forages, due mainly to its adaptability to a wide range of soil and climatic conditions. However, sweet clover possesses some undesirable features which have tended to restrict its use. One of the main problems is the well known hard-seeded characteristic of this crop, caused by the impermeability of the seed coat to water.

A program to develop a permeable-seeded strain was initiated in 1931. The results of this work showed that it was possible to select for permeability, but that it was associated with the occurrence of brownish, sunken, irregular shaped spots on the seed coat. Disease, complex inheritance, and environment have been suggested as either causing or controlling the expression of the spot, and thus permeability in these permeable lines. However, no evidence was obtained as to the cause or nature of the seed coat spots or the number of factors responsible for the inheritance of permeability.

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In this paper an endeavour will be made to answer some of these previously unanswered questions. Several aspects of spotting have been studied, and will be considered under the following headings: inheritance of spotting, environmental effects on spotting, transmission of spotting by graftage, and the histological study of the spot.

For sake of clarity in this paper, the terms permeability and spotting will be used interchangeably to indicate permeability of the seed coat.

LITERATURE REVIEW

Inheritance of Spotting

In the course of a selection program, designed to develop permeable seeded strains, Stevenson (13) first noted the occurrence of brownish spots on the seeds from some plants in highly permeable lines. By treatment with osmic acid he demonstrated that water entry into the seeds took place at these discoloured areas. However, he stated that permeable seeds within these highly permeable lines were not confined to those which possessed the brownish areas.

White and Stevenson (16) in a later paper reported that, although no specific effort was made to select plants producing spotted seed, "through selection of the most highly permeable plants in the lines of highest average permeability, unconscious selection has resulted for lines producing a very high proportion of spotted seeds". Further, they demonstrated the association of the spot with permeability by finding a correlation of + 0.96 between estimated permeability based on the percentage of seeds spotted and actual permeability. They showed further that seeds having such spots were 96.1 per cent permeable, while non-spotted seeds from the same plants were only 11.8 per cent permeable. Thus it was clearly illustrated that high permeability of spotted lines was dependent upon the occurrence of brownish coloured spots on the seed coat.

The inheritance of permeability was studied by White and Stevenson (16) in crosses of the S-30-11 permeable-seeded line with the impermeable-seeded varieties Pioneer, Alpha, Redfield Yellow and a selection, S-268. They found the F_1 plants produced only unspotted seed, and that between 75 and 90 per cent of the F_2 population occurred in the 0-30 per cent permeability class. Further, only the cross, S-30-11 × Pioneer, showed any tendency to a bi-modal distribution, and it was found impossible to fit a genetic ratio to all crosses. From the above evidence it was concluded that the factors for spotting and permeability were recessive, and inherited in a complex manner.

Environmental Effects on Spotting

Anna Lute (6), working with alfalfa, found that individual stems of one plant produced seeds with a different percentage of impermeable seed than other stems of the same plant. She also noted that the seed of the same plants grown in different years had different permeabilities.

In crimson clover a high level of calcium was found by James and Bancroft (5) to significantly increase, while potassium significantly decreased the percentage of hard seed. Phosphorus treatments showed no significant effect, nor were the interactions between phosphorus, potassium and calcium treatments significant.

Soil fertility was found to influence the deposition of pigment in common beans (9) and soybeans (8), but not in sweet clover (13). However, pigment deposition in sweet clover was influenced by other environmental factors, since Stevenson (13) showed that of two seeds in the same pod, both may be mottled, both unmottled, or one mottled and one unmottled.

Transmission of Spotting by Graftage

Evidence that disease might be responsible for spotting has accumulated over the years. In the first place, the brownish spots resembled disease lesions. Secondly, in crosses between permeable and non-permeable strains, the permeability distribution of F_2 progenies, as reported by White and Stevenson (16), could not be interpreted on any genetic basis, whereas many characters are known to be simply inherited in this diploid genus. Thirdly, it has been found that seed grown under selfing bags was considerably less permeable than seed grown under field conditions. Assuming disease was responsible, it was thought that conditions under the selfing bags were less favourable for the disease to develop. Finally, Stevenson (13) had reported bits of tissue resembling mycelium in sections through the spotted areas.

Since what appeared to be pathological symptoms had the characteristics of a virus disease a grafting experiment was undertaken. Smith, in 1891 and 1894, as reported by Bawden (1), appears to be one of the first to demonstrate the transmission of what appeared to be a pathological condition by graftage. Since that time many virus diseases have been demonstrated as transmissible by grafting.

Histological Study of the Spot

Many workers have reported on the structure of the seed coat of legumes. Sweet clover seed has been of particular interest due to the economic importance of the crop and the high degree of impermeability of the seed. Malpighi was one of the early workers who, as cited by Pammel (10), gave the first account of sweet clover seed coats. The palisade-like layer of cells has been named the Malpighian layer in recognition of Malpighi's contribution to the basic research of the seed coat. Later workers such as Coe and Martin (2), Hamly (3), Martin and Watt (7) and Stevenson (13) studied the sweet clover seed coats mainly to locate and explain the tissue responsible for impermeability.

All workers agree that the impermeable layer is located in the outer regions of the seed coat. The seed coat is rather complex (Figure 1). Starting from the outermost layer there is first a thin cuticle and sub-cuticle. Imbedded in this sub-cuticle are the caps and domes of the Malpighian cells. These cells are closely packed, radially placed, and columnar in shape. They are divided, on the basis of refractive differences, into three parts, domes or caps, the light line, and the basal part containing the cell lumen and protoplasmic contents. It is in these cells that most workers locate the impermeable layer. Interior to this layer lie the osteosclerid cells with broad bases and lateral walls thickened in ridges. Under this single row of cells are one or more layers of cells with large lumina,

generally considered to be nutrient tissue. Between the seed coat proper and the cotyledons lie one to several layers of endosperm cells, including an aleurone layer.

Although it is agreed that the impermeable layer lies at the outer end of the Malpighian cells, there is disagreement as to the particular structure involved and as to the chemical make-up of the various layers. Rees (11) and Hamly (3) attributed hardness to the tightly compressed caps of the Malpighian cells. However, Rees (11) reported them as being cuticularized and Hamly (3) classified them as suberized. Coe and Martin (2), and Stevenson (13), on the other hand, attribute impermeability to the light line.

Stevenson (13) reported on observations of sections through the spotted seeds. He found that in the spotted areas the cells were somewhat disorganized, the light line was indistinct, the Malpighian cells shorter, and the osteosclerid cells almost collapsed. In some sections bits of tissue resembling mycelia were observed.

METHODS AND MATERIALS

Inheritance of Spotting

Of the 25 plants selected in 1931 with 10 to 100 per cent permeability, only 5 gave rise to lines which were sufficiently normal in vegetative and reproductive characters to be maintained after one or two inbred generations. After continued selection for high permeability, the 5 strains were reduced to 2, the original selection numbers being S-28-5b and S-30-11. The S-30-11 strain carried an undesirable gene or genes for semi-dwarf habit of growth, and for this reason was discarded in 1943. However, the permeable genes it possessed were carried along in selections S-42-$\overline{16}$, S-42-$\overline{33}$ and S-42-$\overline{34}$ which were obtained from an F_2 of the cross, S-30-11 $\times$ Pioneer, as reported by White and Stevenson (16).

In order to determine the inheritance of spotting, plants of permeable strains S-42-$\overline{33}$, S-42-$\overline{34}$ and S-28-5b were used as male parents in crosses with plants of the Pioneer variety which is non-permeable and low in free coumarin (14). These crosses were made in the greenhouse in 1946, and the F_1 was selfed in the greenhouse in 1947-48. The F_2 was established in the field in a spaced-plant nursery in 1950. The seed was harvested in 1951 by stripping the seed from the racemes of each plant by hand. Dehulling was done with a corrugated rubber mat and rubber covered block.

F_2 plants were classified as spotted or non-spotted based on examination of the open-pollinated seed of each plant under a low power binocular. If any spotted seeds were found, the plant was classified in the spotted group. Permeability tests were also carried out on the same seed partly to reveal any plants which were incorrectly classified and also to determine whether a genetic ratio could be obtained from permeability tests.

Previous to 1951 permeability was determined by placing 50 seeds with a few drops of water in a glass vial and counting the seeds which had swelled or germinated by the fifth day. In the study reported here it was necessary to test duplicate samples from 2,000 plants in a short time; therefore a faster method was needed. The method used was to count out

two samples of 50 well matured open-pollinated seeds from each plant. From some plants there were less than 100 seeds available, so two samples of 25 seeds were used. The seed was then spread on wet paper towels, and the towels stacked in covered ammunition-boxes, with wet paper towels under and over the stacks. It was found that this method gave results equal in accuracy to the vial or Petri dish methods and enabled quicker counting, eliminated washing vials and plates, took less space and materially reduced the amount of mold. Seeds were left in incubation for four days and the temperature was held at approximately 32° C. during the test. Observations indicate that with increased temperature, counts could be taken sooner.

Environmental Effects on Spotting

Seed grown under three different environments was collected from each permeable-parental strain. Five plants from strain S-28-5b, four from S-42-$\overline{34}$ and three from S-42-$\overline{33}$ were used in the study. In the field each plant was selfed by means of a cotton bag, and cuttings of each plant were established and grown to maturity in the greenhouse. Selfed seed was collected from the cuttings in the greenhouse and from under the cotton selfing bags in the field. These selfed seeds were then compared with each other and with open-pollinated seed from their respective plants in the field. Comparisons were made as to the number of seeds spotted and the degree of spotting as well as on a permeability basis.

A sample of 25 open-pollinated seeds from each of 50 F_2 plants, taken at random from the population having spotted seeds, were examined under a binocular. The number of seeds spotted and the degree of spotting on each was determined. The effect of these variations in spotting on permeability was then tested by running a permeability test on the same 25 seeds.

Transmission of Spotting by Graftage

Twelve permeable-seeded plants of the strain S-42-$\overline{16}$-4-4-2 and 12 Arctic plants were brought into the greenhouse in November, 1948. In December and January, 45 grafts were made using sections of Arctic plants as the scion and S-42-$\overline{16}$-4-4-2 plants as the stock or foster parent. The grafts were made by the cleft method and a thin sheet of rubber was wrapped around the stem at the location of the graft. The grafts proved to be quite simple to make and highly successful in that 30 out of 45 grafts caught.

In the greenhouse, selfed seeds of the scion, stock and plants from which the scions came, were obtained by rolling the flowers between the fingers. Crossed seed was also obtained from crosses of the scion with their stocks and the stocks with their respective scion source plants. In June the Arctic plants and the grafts were set outside the greenhouse to be open-pollinated. All seed obtained was examined for spots. Seeds obtained in the greenhouse were grown in spaced-plant, isolated field plots, in 1948, and open-pollinated seed from these plants was examined for spotting in the winter of 1950-51.

Histological Study of the Spot

Seeds used in the histological study were obtained from the F_2 population and parent plants. For serial sections Craf killing and fixing solution

was used, and the outline by Sass (12) for paraffin embedding was followed. When sectioning mature seeds it was found necessary to soak embedded seeds in water over night, after having first cut one or two sections to allow entry of water.

For the study of individual cells, maceration was accomplished with ammonia and hydrochloric acid, as reported by Hamly (3). Safranin and fast green, as well as hematoxylin, were used as staining agents.

EXPERIMENTAL RESULTS

Inheritance of Spotting

Out of the 4,000 or more F_2 plants established in the first year, only about one-half survived a flood in the spring of the second year. The flooding, and generally cool weather of 1951, tended to retard seed setting, so that in only 1,944 cases was sufficiently well matured seed obtained to classify the plants as to the spotted character.

The results of the classification for the three crosses S-42-$\overline{33}$, S-42-$\overline{34}$ and S-28-5b with Pioneer are shown in Table 1. All three crosses gave a good fit to a three to one ratio. Crosses of S-42-$\overline{33}$ and S-42-$\overline{34}$ with Pioneer gave a Chi square probability of 0.95 to 0.50 while the S-28-5b × Pioneer cross gave a probability of 0.10 to 0.05.

In the latter cross one F_1 parent (C-47-HP-18-6), produced only four spotted-seeded plants out of total of 117. These spotted-seeded plants had a very low number of spotted seeds per plant and permeability was correspondingly low. It is suggested that these plants may have been incorrectly classified into the spotted group, and that the F_1 parent arose from a cross of Pioneer with a non-permeable strain. There is also the possibility that the penetrance of the character was low in these progeny or that the parent plant did not receive some necessary modifying genes for the expression of the spot. When the progeny of this F_1 parent is omitted from the population of the cross S-28-5b × Pioneer, the probability value for Chi square is 0.95 to 0.50 which, as can be seen from Table 1, parallels the other crosses.

TABLE 1.—RESULTS IN F_2 SHOWING SEGREGATION OF SPOTTED AND NON-SPOTTED SEED COATS IN A 3 : 1 RATIO

Cross No.	Parents	Number of F_2 plants			Chi²	P
		Non-spotted	Spotted	Total		
1	Pioneer × S-42-$\overline{33}$	473	160	633	0.0256	0.95–0.50
2	Pioneer × S-42-$\overline{34}$	406	140	546	0.1176	0.95–0.50
3	Pioneer × S-28-5b	634	181	815	3.3868	0.10–0.05
*3a	Pioneer × S-28-5b	521	177	698	0.0472	0.95–0.50
	Combined crosses	1,513	481	1,994	0.8190	0.50–0.20
	*Combined crosses	1,400	477	1,877	0.1322	0.95–0.50

* Progeny of C-47-HP-18-6 is omitted.

TABLE 2.—FREQUENCY DISTRIBUTION FOR PERMEABILITY OF THE F_2 PLANTS CLASSIFIED AS SPOTTED AND NON-SPOTTED GIVING NUMBER AND PER CENT OF POPULATION IN PER CENT PERMEABILITY CLASS

Cross	Classification	Per cent permeability class												Total number of plants	Mean perm.
		0	1 — 9	10 — 19	20 — 29	30 — 39	40 — 49	50 — 59	60 — 69	70 — 79	80 — 89	90 — 99	100		
Pioneer × S-42-$\overline{33}$	*Spotted*														
	Number of plants in	0	6	8	2	3	5	7	10	23	34	54	5	157	74.80
	Per cent of plants in	0	4	5	1	2	3	4	6	14	22	34	3		
	Non-spotted														
	Number of plants in	7	281	132	36	6								462	9.04
	Number of plants in	2	61	29	8	1									
Pioneer × S-42-$\overline{34}$	*Spotted*—														
	Number of plants in	0	3	3	2	1	5	6	3	20	33	25	0	101	73.74
	Per cent of plants in	0	3	3	2	1	5	6	3	20	33	25	0		
	Non-spotted—														
	Number of plants in	8	251	72	28	6								365	8.20
	Per cent of plants in	2	69	20	8	2									
Pioneer × S-28-5b	*Spotted*—														
	Number of plants in	0	8	3	4	3	6	7	7	3	39	63	2	145	76.13
	Per cent of plants in	0	6	2	3	2	4	5	5	2	27	43	1		
	Non-spotted—														
	Number of plants in	36	475	71	17	7								606	5.47
	Number of plants in	6	78	12	3	1									
Combined total of three crosses	*Spotted*—														
	Number of plants in	0	17	14	8	7	16	20	20	46	106	142	7	403	75.04
	Per cent of plants in	0	4	3	2	2	4	5	5	11	26	35	2		
	Non-spotted—														
	Number of plants in	51	1007	275	81	19								1433	7.32
	Per cent of plants in	4	70	19	6	1									
Parents—															
Pioneer	Non-spotted	5	19	9	2									35	5.42
S-42-$\overline{33}$	Spotted									1	4	3	3	11	88.09
S-42-$\overline{34}$	Spotted											3	2	5	98.00
S-28-5b	Spotted									1	4			5	82.00

When the families were pooled and a heterogeneity Chi square calculated they were found to be homogeneous. The heterogeneity Chi square value was 2.7110 and 0.0582 depending on whether the progeny of C-47-HP-18-6 is omitted or not. These values gave a probability of 0.30 to 0.20 and 0.90 to 0.80, respectively, indicating that all families were homogeneous for the character. The total population gave a good fit to a 3 : 1 ratio as shown in Table I. Thus it would appear that spotting is controlled by one major gene inherited in a simple Mendelian manner.

Practically all plants in Table 1 were classified into permeability classes on the basis of the permeability of their seed. The results are given in Table 2. The permeability of seed from plants classified as spotted ranged all the way from the 1-9 per cent group up to the 100 per cent group. The non-spotted plants ranged up to the 30-39 per cent class with a very large percentage of the plants falling into the 1-19 per cent range. These results would indicate that despite the simple inheritance of the character, environment also has a strong influence on the permeability of seeds from spotted plants. It is also interesting to note that in each of the three crosses the percentage of individuals in any one permeability class remains fairly constant, indicating that the environment affected each cross in a similar manner.

It appears that the 39 per cent permeability class would make a satisfactory dividing line between permeable and non-permeable plants. However, the difficulty of using this arbitrary division would be twofold. First, between 8 and 13 per cent of the spotted plants would be included in the non-permeable group. Secondly, the distribution of both groups, as well as the dividing line, may vary from year to year depending on environmental conditions.

Since White and Stevenson (16) reported that the cross between permeable strains and the Pioneer variety was the only cross in which the F_2 population permeability curve could be fitted to any genetic ratio, it was thought there may be some linkage between low free coumarin and spotting. In order to investigate this hypothesis, plants were tested in the summer of 1951 by the micro-colorimetric test for coumarin (4). Linkage could be tested for in only one cross, S-28-5b with Pioneer, as the other two permeable parents had Pioneer in their ancestry. Progenies of four F_1 plants, C-47-HP-18-12, — 14, — 15, — 6 were also excluded. The first three were omitted as no accurate coumarin determination could be obtained due to their advanced state of maturity at the time of the test (15). The progeny of C-47-HP-18-6 was left out since it is doubtful if the F_1 plant was from a permeable × Pioneer cross.

As can be seen from Table 3 the data fit a two-factor ratio satisfactorily indicating no linkage.

Environmental Effects on Spotting

Open-pollinated seed was compared with seed from cuttings grown in the greenhouse, and seed produced under selfing bags in the field.

Spotting and permeability were markedly reduced under selfing bags. However, selfed seed from the greenhouse did not show any significant decrease in the percentage of spotted seeds when compared with open seed

TABLE 3.—SEGREGATION OF COUMARIN AND SEED SPOTTING IN THE F_2 OF THE CROSS BETWEEN S-28-5b PERMEABLE STRAIN AND THE LOW FREE COUMARIN VARIETY PIONEER

Expected ratio	F_2 phenotype	No. of plants	$\frac{(O-C)^2}{C}$	P
9	Non-spotted, high coumarin	226	0.4447	
3	Non-spotted, low coumarin	80	0.0198	
3	Spotted, high coumarin	79	0.0007	
1	Spotted, low coumarin	35	2.9166	
		X^2	3.3818	0.50–0.10

from the field. It was found, however, that the degree of spotting decreased from open-pollinated seed, to greenhouse selfed, to selfed seed produced under selfing bags in the field.

To establish further the degree to which permeability depends on the occurrence and the degree of the spot, the following study was undertaken. Twenty-five seeds from each of 50 spotted plants, taken at random, were classified as to the number of seeds spotted, as well as to the degree of spotting. A permeability test on these same seeds showed a correlation of + 0.95 between spotting and permeability. However, there was little, if any, relationship between the degree of spotting and permeability as can be seen in Table 4.

Only in one case was there a significant difference between spotting and permeability and that occurred in selfed seeds obtained in the greenhouse. However, in this case, due to an error, the seeds used in the permeability test were not necessarily the same 25 seeds which were examined for the number of spotted seeds.

Even though there were considerable differences in fertility and texture of the soil used to grow the cuttings in the greenhouse and the soil in the field, no significant difference was found in the number of seeds spotted. Examinations of double-seeded pods showed that there was a micro-environmental effect as one or both or neither seeds were spotted. In the majority of cases, however, either both seeds were spotted or both unspotted.

Transmission of Spotting by Graftage

In order to determine whether or not a transmissible disease was responsible for spotting a graftage study was undertaken prior to the inheritance study. It is evident that the spotted character occurs in the maternal tissue, i.e., the seed coat. Consequently, if spotting were due to a disease transmitted systemically through the tissues of the plant, then it should be carried throughout the scion and should appear on either the scion seed or in plants grown from that seed.

Observations were made on selfed and open-pollinated seed obtained inside and outside the greenhouse in 1949. Open-pollinated seeds from permeable stocks were almost completely spotted, while open-pollinated,

TABLE 4.—COMPARISON OF NUMBER OF SEEDS SPOTTED, THE DEGREE OF SPOTTING AND PERMEABILITY OF OPEN-POLLINATED SEED AND SELFED SEEDS PRODUCED UNDER TWO CONDITIONS. COMPARISON BASED ON SAMPLES OF TWENTY-FIVE SEEDS

Plant	Per 25 seeds		Degree of spotting*										Mean
	No. spotted	No. perm.	1	2	3	4	5	6	7	8	9	10	
Field Open-pollinated													
A	21	21	4	4	4	2	3	1	3				3.5
B	16	20	6	3		4	2				1		3.0
C	24	24	1	3	3	2	2	2	5	4	2		5.4
D	24	25	2	2	1	2	7	4	2	1	3		5.2
E	25	25			1	5	4	3	4	5	3		6.2
F	25	25	1	2	3	8	6	2			1	1	4.4
G	22	22	4	6	4	4	3	1					2.9
H	25	21	1	7	4	8	3		1	1			3.5
I	11	12	3	3	1	4							2.5
J	24	22	7	6	3	4	2	2					2.7
K	24	24	2	5	5	8	2	1	1				3.4
L	24	23	4	4	3	3	4	3	2	1			3.8
Mean	21.3	22.0											3.87
Greenhouse Selfed Seed													
A	20	20	5	9	4	1		1					2.2
B	16	15	3	4	3	3	2		1				3.0
C	24	24		3	3	5	3	4		1	1	1	4.5
D	24	23	2	7	1	5	2	3		1	3		4.2
E	23	23		3	2	3	4	4	3	2	2		5.3
F	25	24		5	5	8	2	4	1				3.9
G	10	8	4	4	2								1.8
H	20	14	3	8	6	3							2.4
I	13	8	7	3	2	1							1.7
J	21	12	7	4	3	5	2						2.5
K	24	21	3	6	5	7	2		1				3.1
L	20	22	2	5	1								1.7
Mean	20.0	17.8											2.67
Selfed Seed from Selfing Bags													
A	8	8	2	2	2	2							2.5
B	0	0											0.0
C	8	7	3	2	1	1	1						2.3
D													
E	10	9	9	1	7	1	1						2.2
F	11	11	2	4	4	1							2.3
G	0	0											0.0
H	4	2	3		1								1.5
I													
J	3	6	3										1.0
K	3	3	1	1		1							2.3
L	3	3	3										1.0
Mean	5.0	4.9											1.51

* Spot size increasing from 1 to 10.

non-permeable Arctic scion source plants produced only unspotted seed. Of the 3,701 open-pollinated seeds obtained from grafts of Arctic scions on permeable stocks only four seeds were spotted. Similarly only one of the 1,778 greenhouse selfed seeds, obtained from the Arctic graft was spotted. The extremely low frequency of spotted seed obtained from the grafts suggests that they were probably mechanically mixed in harvesting.

In 1950, open-pollinated seed was harvested from F_1 plants grown under isolation. These plants resulted from crosses between scions and their respective stocks and the permeable stocks with the Arctic scion-sources. Open seed was also harvested from selfed progenies of Arctic scions, their sources and the permeable stocks. All seed was then examined from each plant and the plant classified as spotted or non-spotted.

Selfed progenies of permeable seeded parent stocks produced 5 plants out of a total of 91 which were classified as non-spotted. These 5 plants could have arisen from mechanical mixing in harvesting selfed seed from the stock or they may have been rogues which volunteered where a plant was missing.

Of the open seed of selfed progenies from Arctic plants used as graft sources no spotted seeds were found. Since these plants had no contact with permeable plants the results obtained were to be expected.

Selfed seed from Arctic grafts produced 878 plants with unspotted seeds to one plant with spotted seeds. The high degree of spotting on the seed of this plant suggests strongly that it arose from mechanical mixing of a permeable seed from the stock while collecting selfed seed from the scion.

When the permeable stocks, used as females, were crossed with Arctic scion-source plants, 42 to 310 F_1 progenies were spotted; however, these plants were probably selfs since no spotted seeds were found on F_1 plants from the reciprocal cross of the permeable stock with its scion.

It would be expected that, if disease was the cause of spotting, the F_1 progenies of the cross between the scion and stock would be the most likely to produce spotted seed. The results, however, are the exact opposite. All data from the graftage experiment indicate that if the lesion is caused by disease it is not transmissible systemically to non-permeable material. Moreover, the disease was not transmitted through the pollen.

Thus, the graftage experiments support the hypothesis that spotting is controlled by a genetic character inherited as a recessive.

Histological Study of the Spot

Spotted seed was examined to determinate the position of the spot on the seed coat, in relation to internal parts of the seed. Examinations showed that in nearly all cases the spot began at the hypocotyl and extended along the seed coat for one-quarter to one-half the length of the cotyledon. Various typical spot formations are shown in Figure 2. Examination of seeds from multiple seeded pods revealed that spots did not occur on areas of the seed coats that were adjacent. However, when there was only one seed per pod, spots generally were found on both sides of the seed. When this occurred, there was generally a ridge of normal, non-spotted tissue dividing the spot in two. This ridge extended down the back of the seed and its location corresponded to the space between the cotyledons (Figure 2).

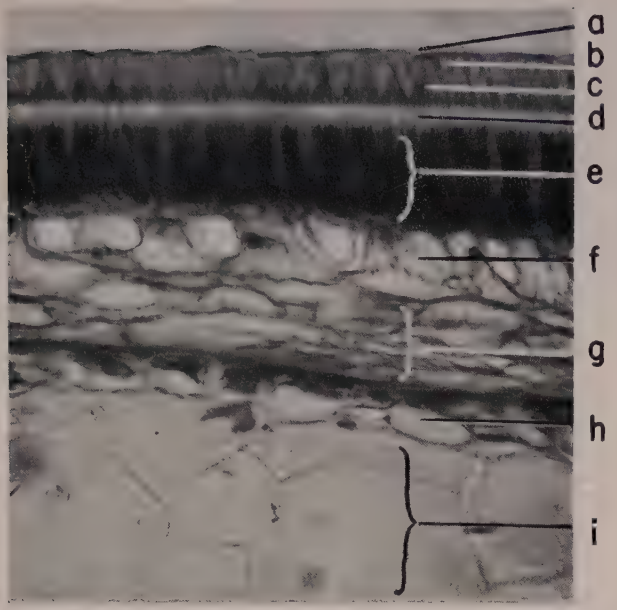

FIGURE 1. Radical section of seed coat; *a*, cuticle; *b*, subcuticle layer; *c*, caps of Malpighian cells; *d*, light line; *e*, basial portion of Malpighian cells; *f*, osteosclerid cells; *g*, nutrient layer; *h*, aleurone layer; *i*, inner layers of endosperm. (× 570).

FIGURE 2. Spotted seeds typical of those produced by permeable plants, seeds in bottom row showing dorsal surface. (× 10).

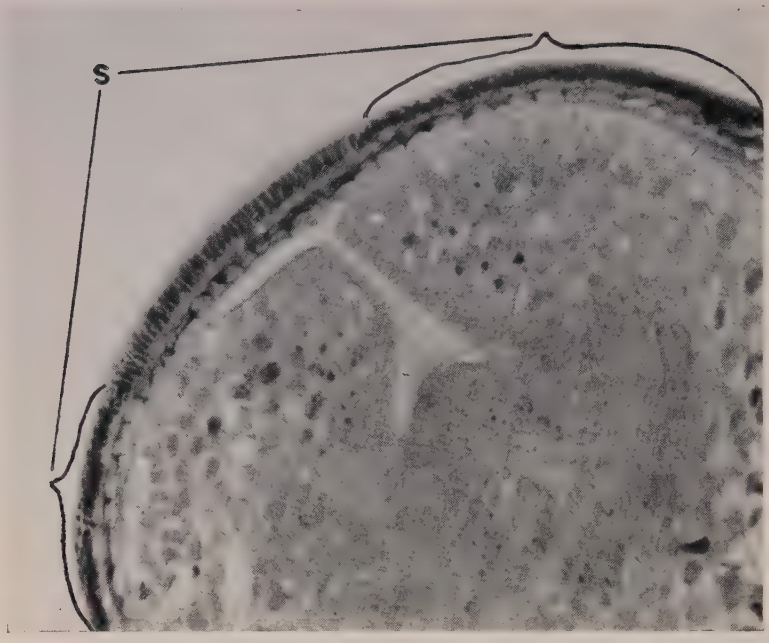

FIGURE 3. Cross section of spotted seed showing spots where cotyledons press against the seed coat. (× 150).

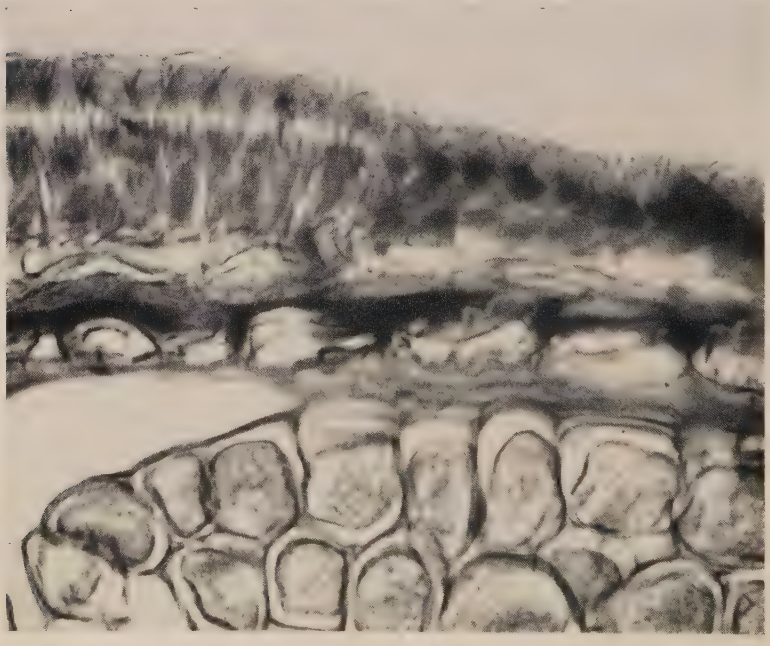

FIGURE 4. Section through seed coat showing normal area on left and spotted section on right. (× 560).

The spot was not distinguishable until the seed matured, and the pigment was deposited. Serial sections were made of seeds at 10, 15, 18, 23 and 28 days after pollination. No spots were observed in serial sections until the 28-day stage. However, it is possible that spots were overlooked, since only two or three seeds at each stage of development were sectioned. In all observations of spotted areas no mycelium was observed.

Sections through the spotted areas (Figure 3) and macerated material from spotted seed coats (Figure 5) showed the Malpighian cells, when compared with the normal (Figure 6), were short, distorted and bent. The light line had all but disappeared while the lumen was completely filled with a brown pigment.

In sections through the spot tissue observation showed the osteosclerid cells to be flattened and either crushed or collapsed. Seed coat nutrient tissue was not present and of the endosperm only the aleurone remained in spotted areas. (Figures 3 and 4).

Sections also showed that cotyledons pressed against the seed coat at one or more places in the spotted area (Figures 3 and 4). Untreated seeds were also cut longitudinally. While non-spotted seed had considerable nutritive tissue between the top of the cotyledon and the seed coat, spotted seed had little if any tissue between the cotyledon and the spotted area of the seed coat.

DISCUSSION

The simple inheritance of spotting and permeability appears to be established by the results of the genetic study. However, a close observation of White and Stevenson's results (16) with other crosses and in the development of inbred permeable seeded lines, indicates that one or more modifying genes may be involved. For a simply inherited character extensive inbreeding should not be necessary; it is possible that there are modifying factors, or that the penetrance is low, or a combination of these phenomena. It may be difficult to select for these modifying genes because of low heritability, resulting in some of the modifiers becoming fixed only after continuous generations of inbreeding.

In homozygous permeable inbred lines a very low percentage of individuals have permeability ratings lower than 60 per cent. However, in the F_2 populations (Table 2) 18 per cent of the spotted plants had a permeability below 60 per cent. Probably the most conclusive evidence is that White and Stevenson (16) were able to select from the cross S-30-11 × Pioneer, lines with higher permeability than could be obtained by continued selections in the S-30-11 line. This indicates that some gene or genes for permeability were obtained from the out-cross.

Since Pioneer appears to be carrying some modifying gene or genes for permeability, and since Pioneer was developed by an inbreeding program involving mainly selection of low free coumarin types, it is logical to expect there would be linkage between these two genes. The linkage study, however, shows no linkage between the main gene for permeability and that for low free coumarin. On the other hand, this does not establish that there is no linkage between modifying genes and the gene for low free coumarin. It may also be that the F_1 parent C-47-HP-18-6 did not receive the modifying factors because of broken linkage thus producing only four

spotted progenies out of a total of 117. Although the inheritance of spotting is controlled by one major gene the threshold of expression may be a fine balance between environment and one or more modifying genes.

It is obvious from the results of the environment and genetic studies that environment plays an important role in determining the penetrance and expressivity of the character. This is indicated by the percentage of spotted seeds and the degree of spotting in seed from a plant homozyous for the spotted character. It was shown that there was a micro-environmental effect but the controlling environmental factor affecting spotting could not be determined. The spot appears to be better expressed under good growing conditions.

The practically complete absence of spotting on seed from the grafts is good evidence that spotting is not caused by a virus disease. If a disease is the cause then it is either non-transmissible by grafting or the Arctic plants used as scions must have been resistant to it. The possibility that Arctic plants used as scions were resistant to the disease is not likely since permeable lines were originally selected from the Arctic variety, and nine unrelated Arctic plants were used as scion sources. Thus, with such genetic diversity there is little likelihood that all plants would be resistant. Since a virus disease should have been transmissible by graftage and since in the histological investigations no mycelium or other positive indication of disease was observed, it is concluded that spotting is not due to disease.

Although the graftage study contributed little positive information on the cause of the spot, the histological observations of the spot suggest the following possible causes:

(*a*) The embryo may be feeding on seed coat tissue or nutrient intended for the seed coat, causing disorganization of the cells of the seed coat.
(*b*) The cotyledons may be growing too large for the seed coat in certain areas thus crushing and distorting the seed coat cells.
(*c*) Certain portions of the seed coat may be smaller than normal in relation to cotyledon size.

While there is not sufficient evidence to prove or disprove the theory of embryo feeding on the seed coat nutrient, observations show that an aleurone layer is present at the spot. In addition to the aleurone, there is sufficient endosperm in the seed to cause, upon water entry, sufficient swelling to release the embryo from the seed coat. These observations, while not disproving the theory, make it less probable than the other two.

The second and third hypotheses can hardly be separated from one another as it is impossible to say, without further tests, whether it is the seed coat that is smaller or the cotyledons that are larger than in normal non-spotted seed. The consistency with which the spot follows the cotyledon pattern under the seed coat suggests strongly that the cotyledons are involved. However, since the embryos resulting from open-pollination will not often grown into spotted-seeded plants, due to the recessiveness of the character, it is evident that maternal tissue would have control over cotyledon size. Such a control would not be unexpected as indicated by unpublished results on sweet clover seed weight studies at the Forage Crops Laboratory, Saskatoon. These studies have shown that by selection plants having seed weight differences of 100 per cent or more can be obtained. This would indicate that seed weight is an inherited charac-

FIGURE 5. Distorted Malpighian cells from spotted area. The cell at the lower right is an osteosclerid. (× 1120).

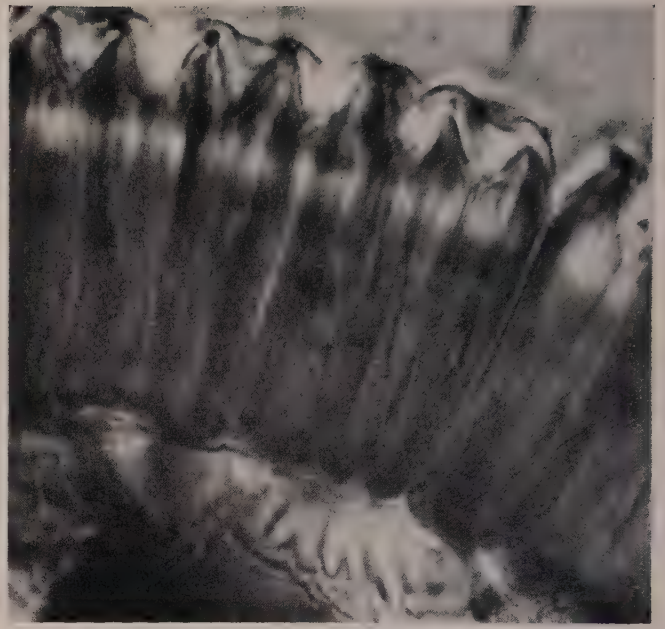

FIGURE 6. Normal Malpighian cells. (× 1120).

teristic which can be selected for and that the mother plant has some control over seed size. The seed coat, which is maternal tissue, may be the cause of the spot due to differential growth rates of the seed coat and cotyledons.

The evidence for these hypotheses is inconclusive. It is felt, however, that they may serve as basis for future study. One of the more important tasks that should be undertaken is to section a large number of spotted seeds at various stages of maturity. This study would not only establish the stage of embryo development at which spotting occurs but also whether a crushing action takes place in the spotted areas. Since the dependence of permeability on spotting is clearly established, and since spotting is now known to be simply inherited, there should be a quick development of new permeable-seeded lines. As these lines are combined to obtain other good qualities, a broad genetic base will become available for the development of sweet clover varieties having permeable seed as well as other desirable characteristics.

ACKNOWLEDGMENTS

The writers wish to acknowledge, with thanks, the contribution of R. G. Savage, now Assistant Superintendent of the Experimental Station at Prince George, British Columbia, who made the grafts and crosses from which the data herein were reported. Thanks are also due to R. A. Ingalls and his staff of the Saskatoon office of the Production Service, Plant Products Division, who aided in counting the many seed samples.

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PLANT PIGMENTS AS INTERNAL INDICATORS OF DIGESTIBILITY OF DRY MATTER OF PASTURE HERBAGE[1]

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[Received for publication April 26, 1954]

ABSTRACT

Four digestibility trials were conducted to evaluate the plant pigment (chromogen) method of determining dry matter digestibility of pasture herbage. Grass was cut from a native permanent pasture and dehydrated artificially to approximately 90 per cent dry matter. In one trial with sheep the digestibility coefficients obtained by both the chromogen ratio and the chromogen formula methods of Reid *et al.* were practically identical to those obtained by the conventional total faecal collection technique. In three trials with steers a slight wavelength adjustment was necessary to obtain optimum agreement between the three methods. It was concluded that the plant pigments may be useful indicators of the dry matter digestibility of pasture herbage but various grazing conditions should be investigated further.

INTRODUCTION

The study of pasture productivity with freely grazing animals has been handicapped by lack of simple and accurate experimental techniques. The following procedure has been followed in this laboratory. One group of sheep was fitted with faeces collection bags and allowed to graze freely. An adjacent pasture area was clipped, the forage dehydrated, and digestibility coefficients were determined by the total collection method. The feed consumption of the freely grazing animals was estimated from their total faecal output by applying the digestibility coefficients obtained in the laboratory.

This indirect method was laborious and cumbersome. Moreover, the validity of the results depends on the validity of two assumptions, viz., that artificial dehydration does not affect the digestibility of the forage and that the pasture clippings are truly representative of the herbage consumed by the freely grazing animals.

Of various methods that have been proposed for overcoming these objections, the indicator method of Reid *et al.* (7) appeared most promising. In this method forage and faeces are extracted with 85 per cent acetone and the pigment (chromogen) concentration estimated spectrophotometrically at 406 mμ. The dry matter digestibility of the forage is then calculated from the following formula:

$$\text{Digestibility Coefficient} = 100 - 100\,\frac{(\text{pigment concentration in feed})}{(\text{pigment concentration in faeces})}.$$

This method obviates conventional digestibility trials in the laboratory.

In more recent work, Reid *et al.* (8) made the observation that regardless of the botanical composition, stage of maturity and degree of digesti-

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bility of the particular species and mixtures investigated, a definite relationship existed between the chromogen concentration of the faeces and of the consumed forage. That is, pigment concentration in the faeces having been determined, pigment concentration in the feed could be calculated and hence the digestibility estimated. The formula proposed for this purpose would be most useful if it applied to Canadian pastures, for its use avoids the conventional digestibility trials and overcomes the forage sampling problem. Reid* stated that the formula has been successfully applied in trials carried out during various pasture seasons.

Kane *et al.* (5, 6) compared various digestion trial techniques with dairy cattle and observed that the plant pigment formula method was satisfactory but that the pigment ratio method did not provide a valid index of digestibility because the recovery of chromogens in faeces was 129 per cent. Cook and Harris (2) reported little success with the chromogen technique in digestibility studies of desert range plants with grazing sheep.

Preliminary investigations in the Ottawa laborarory showed that the plant pigment technique was not applicable to feedstuffs which contain small amounts of chromogens, such as grains, corn silage, and barn-cured hay which contained small amounts of chromogens. Davidson (3, 4) and Smart *et al.* (9) studied the nature of the pigments extracted from herbage and faeces by 85 per cent acetone. They noted that the total absorption at 406 mμ was a summation of that due to several pigments in the forage and their degradation products through the alimentary canal. They warned that the chromogen method should be treated with caution until its validity had been proved for any given type of pasture herbage.

The experiments reported here were undertaken to investigate the chromogen technique with cattle and sheep in one phase of the study of the productivity of Canadian pastures.

EXPERIMENTAL

Four wethers (Shropshire male × Leicester female) and four Grade Shorthorn steers were used in these experiments. The grass was cut from a typical permanent pasture. The botanical composition was approximately as follows: timothy *Phleum pratense* L. 30, Kentucky blue grass *Poa pratensis* L. 15, Canada blue grass *Poa compressa* L. 15, white clover *Trifolium repens* L. 20, common vetch *Vicia sativa* L. 10, and weeds 10.

A description of the herbage used in each trial is given below:

Trial with Sheep

First cut made early in the season when the grass was approximately 5 to 6 inches high.

Trials with Steers

Trial 1—The grass was approximately 8 to 10 inches high.

Trial 2—Approximately 50 per cent of the timothy and blue grasses was headed out.

Trial 3—The forage consisted of the aftermath from the same area yielding the forage for Trial 1.

*Personal communication.

In each case the cut grass was artificially dried in a forced draft oil-fired dehydrator to approximately 90 per cent dry matter.

The dry matter digestibility of the various forages was determined by the conventional total collection technique according to the procedure adopted in this laboratory and described in detail in previous publications (10, 11). This procedure was slightly modified in the sheep experiments when the animals were fed the dehydrated forage for a 7-day preliminary period and a 7-day collection period.

The grass and the faeces were sampled and analysed for dry matter and chromogen at four and five intervals during the collection period in the sheep and steer trials, respectively.

The extraction of the pigments was carried out according to the procedure described by Reid *et al.* (7, 8). The optical densities of the various extracts were recorded at ten different wavelengths between 395 and 445 mμ, using a Beckman Model B spectrophotometer. This instrument was calibrated against the Beckman Model DU which had been previously calibrated against the yellow line of sodium.

The dry matter digestibility obtained by the chromogen ratio method was calculated according to the following formula:

Coefficient of digestibility of dry matter $= 100 - 100 \frac{a}{b}$ where a = optical density of an extract of 1 gram of dry feed diluted to 1 litre, and b = optical density of an extract of 1 gram of dry faeces diluted to 1 litre.

The dry matter digestibility coefficients were also calculated by the chromogen formula reported by Reid *et al.* (8).

RESULTS AND DISCUSSION

The absorption spectra of the acetone extracts of faeces were similar to those reported by Reid *et al.* (7). The absorption spectra of the grass extracts, however, differed slightly since they showed no comparable maxima around 430 mμ. It is probable that some of the pigments absorbing light in that region of the spectrum were destroyed during dehydration.

TABLE 1.—THE APPARENT DRY MATTER DIGESTIBILITY OF DEHYDRATED PASTURE CLIPPINGS DETERMINED BY VARIOUS METHODS WITH SHEEP

(Coefficients in per cent)

Animal	Conventional ratio	Chromogen ratio at 406 mμ	Chromogen formula at 406 mμ
1	72.1	73.0	72.5
2	75.0	73.8	72.9
3	73.3	74.4	73.0
4	72.3	75.3	73.5
Average	73.2	74.1	73.0

TABLE 2.—THE APPARENT DRY MATTER DIGESTIBILITY OF DEHYDRATED PASTURE CLIPPINGS DETERMINED BY VARIOUS METHODS WITH STEERS

(Coefficients in per cent)

Trial	Animal	Conventional ratio	Chromogen ratio at 404 mμ	Chromogen formula at 404 mμ
I	1	68.2	72.3	68.5
	2	67.9	71.6	68.2
	3	67.8	70.4	67.6
	4	66.9	70.2	67.6
Average		67.7	71.1	68.0
II	1	62.1	61.7	61.1
	2	60.7	61.5	61.0
	3	59.6	60.5	60.7
	4	62.1	62.6	61.4
Average		61.1	61.6	61.1
III	1	64.1	63.1	62.9
	2	60.8	61.8	62.4
	3	61.1	61.6	62.3
	4	62.1	61.6	62.4
Average		62.0	62.0	62.5

A comparison of the coefficients of digestibility obtained by the various methods investigated in these experiments can be made from the data in Tables 1 and 2. There was good agreement between the coefficients obtained by the total collection technique and by both chromogen methods. The coefficients given in Table 2 were obtained from the optical densities recorded at 404 mμ instead of 406 mμ as suggested by Reid *et al.* (7, 8). The coefficients calculated by both the chromogen ratio and the chromogen formula technique from readings at 406 mμ were higher by nearly 4 per cent than those obtained conventionally. This difference was statistically significant at $P = 0.01$. In the experiment with sheep, however, the coefficients calculated by the indicator methods from readings at 406 mμ were practically identical to those obtained by the conventional method. It is interesting to note that the chromogen formula developed by Reid *et al.* (8) from data obtained with steers was equally applicable in one of these experiments with sheep.

The limited data of these experiments do not satisfactorily explain the necessity for the wavelength adjustment in the trials with steers. However, it was noted that the chromogen content of the dehydrated grass fed to steers was lower than that of the younger grass fed to sheep. Brisson *et al.*

(1) showed that the critical wavelength for optimum results with the chromogen method shifted to the lower wavelengths when the pigment content of the forage decreased due to increasing stage of maturity. Therefore, it is possible that the wavelength adjustment necessary for agreement between the indicator method and the conventional method, in the experiments with steers, was due fundamentally to the stage of maturity at which the grass was cut.

It is concluded that Reid's chromogen method will prove useful in the study of productivity of pasture in this part of Canada. Further studies are in progress to evaluate the method under different grazing conditions.

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THE INFLUENCE OF TEMPERATURE ON THE TIME REQUIRED TO CONTROL LOOSE SMUT OF BARLEY BY MEANS OF THE SPERGON OR WATER-SOAK TREATMENTS[1]

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[Received for publication April 29, 1954]

ABSTRACT

The results of experiments reported in this paper show that loose smut of barley [*Ustilago nuda* (Jens.) Rost.] can be controlled by soaking the seed in a 0.2 per cent suspension of Spergon at 72° F. for 48 hours. Control was nearly complete when the seed was soaked at 62° F. for 72 hours.

When water alone is used, complete control can be secured at any temperature from 66° F. to 86° F. if the duration of the soaking is adjusted to suit the temperature. At a temperature of 40° F., soaking the seed for 90 hours caused only a slight reduction in the amount of smut. Soaking it at 55° F. and at 62° F. for 90 hours achieved partial control. Soaking it at 66° F. for 80 hours, 72° F. for 65 hours, 76° F. for 55 hours, or 86° F. for 35 hours all appear to be suitable combinations of temperature and time to give complete control of the disease with a minimum of injury to the seed.

INTRODUCTION

In 1888 Jensen (2) discovered that the loose smuts of wheat and barley could be eliminated by treating the seed with hot water. For over 60 years this was the only reliable treatment known for the control of these diseases. The hot water treatment is effective, but it is difficult to apply. This fact has led to a search for a simpler treatment for the control of loose smut.

In 1933 Hanna and Popp (1) reported the results of experiments on the control of loose smut of wheat with hot water, hot air, and with various chemicals in aqueous solution. One of the chemicals, potassium iodide, gave partial control of the disease and was considered worthy of further testing.

In 1951 Tyner (5) described the effects of soaking infected barley seed in a variety of chemical solutions and of treating it with supersonic vibrations. Of the chemicals tested, Spergon (tetrachloro-para-benzoquinone) proved to be the most effective. The longest period of immersion tried was 40 hours and the control was almost complete. Later Tyner and Russell (6) published the results of tests in which satisfactory control was achieved by soaking infected seed in a weak suspension of Spergon for longer periods.

More recently both Tyner (7) and Russell (3, 4) have reported the control of loose smut by soaking infected seed in water, without the addition of any chemical substance.

Preliminary experiments reported by these authors (7, 4) showed that neither the Spergon treatment nor the water soak treatment would give satisfactory control of loose smut if applied at 40° to 60° F. for periods comparable to those required to give control at 70° F. It is important that the temperature needed to ensure control of the smut within any specified period should be more clearly defined. The present paper gives the results of experiments designed to clarify this point.

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An experiment, reported previously (4), showed that the application of the water-soak treatment in closed flasks eliminated the smut more quickly than did its application at the same temperature in open dishes.

MATERIALS AND METHODS

Several commonly grown varieties of barley were used in these experiments. All samples were naturally infected with *Ustilago nuda*. The seed was treated by soaking it in either a 0.2 per cent suspension of Spergon in water, or in water alone, for certain specified periods at controlled temperatures. As the temperature was increased the period of immersion was shortened. The Spergon treatment was applied to the seed in open vessels. The water soak treatment was applied either in stoppered Erlenmeyer flasks, filled to the top with water so as to exclude the air, or in open glass dishes, so that the surface of the water was exposed to the air. Unless otherwise stated, the treated seed was sown in the field.

EXPERIMENTAL RESULTS

In the first experiment Newal barley was treated with Spergon. At temperatures of 55° F. and 62° F. the treatment was applied for periods of 48, 52, 56, 64, 68, and 72 hours. At temperatures of 72° F. and 76° F. three periods were used, namely 48, 52 and 56 hours. Data on the total number of heads and the number smutted, after these various treatments, are shown in Table 1.

TABLE 1.—THE EFFECT OF TEMPERATURE AND TIME ON THE CONTROL OF LOOSE SMUT IN NEWAL BARLEY BY SOAKING THE SEED IN SUSPENSIONS OF SPERGON (0.2%)

Temperature	Hours immersed	Total heads	Smutted heads	Percentage of smutted heads
° F.				
55	48	1124	58	5.2
	52	1172	28	2.4
	56	1132	33	2.9
	64	1112	18	1.6
	68	1076	17	1.6
	72	1076	15	1.4
62	48	1156	46	4.0
	52	1092	36	3.3
	56	1100	45	4.1
	64	1092	22	2.0
	68	1004	8	0.8
	72	956	3	0.3
72	48	980	0	0.0
	52	848	1	0.1
	56	732	0	0.0
76	48	1020	0	0.0
	52	888	0	0.0
	56	576	0	0.0
Control	—	1260	132	10.5

The control of loose smut was incomplete and unsatisfactory when the seed was treated in Spergon suspension at 55° or 62° F. for the periods used. However, at these temperatures the effectiveness of the treatment improved with increases in the period of immersion. At 72° F. and 76° F. smut control was essentially complete for all periods of immersion employed. Greater or lesser reductions in stand were noted for all variations of the treatment.

In the second experiment the barley was treated in open vessels by the water soak method. The same temperatures were employed, but the periods of immersion were longer. The results are shown in Table 2.

TABLE 2.—THE EFFECT OF TEMPERATURE AND TIME ON THE CONTROL OF LOOSE SMUT IN NEWAL BARLEY BY SOAKING THE SEED IN WATER

Temperature	Hours immersed	Total heads	Smutted heads	Percentage of heads smutted
° F.				
55	64	1132	65	5.7
	68	1180	76	6.4
	72	1248	67	5.4
	90	1172	40	3.4
62	64	1292	77	6.0
	68	1284	77	6.0
	72	1416	48	3.4
	90	1376	43	3.1
72	64	1256	0	0.0
	68	1372	0	0.0
	72	1356	0	0.0
	90	1272	0	0.0
76	64	1168	0	0.0
	68	1060	0	0.0
	72	988	0	0.0
	90	288	0	0.0
Control	0	1236	112	9.1

Some reduction in smut resulted from soaking the seed at 55° F. or 62° F. but even after treatment for 90 hours smut control was unsatisfactory. At the higher temperatures control of the smut was complete. Little or no reduction in stand occurred except in the rows treated at 76° F. for the longer periods.

One experiment relating to the influence of temperature on the control of loose smut was conducted in the greenhouse. Two heavily infected samples of barley were treated by the water soak method. One portion of each was sown without treatment and three other portions were treated for 60 hours at temperatures of 60°, 70° and 80° F., respectively. The seed was treated in corked Erlenmeyer flasks. The results of this experiment are shown in Table 3.

TABLE 3.—THE INFLUENCE OF TEMPERATURE ON THE CONTROL OF LOOSE SMUT WHEN THE SEED WAS TREATED BY SOAKING IT IN WATER FOR 60 HOURS

Variety	Hours immersed	Temperature	Total number of heads	Percentage smutted	Yields*
		° F.			
Montcalm	None	—	314	19.0	100
Montcalm	60	60	308	13.3	105
Montcalm	60	70	257	3.9	97
Montcalm	60	80	193	0.0	76
Warrior	None	—	306	23.2	100
Warrior	60	60	305	15.7	109
Warrior	60	70	267	2.2	111
Warrior	60	80	189	0.0	80

* This indicates the number of healthy heads expressed as a percentage of the number of healthy heads in the check rows.

In this experiment, treatment in water at 60° F. for 60 hours produced only a moderate reduction in smut infection. Treatment for the same length of time at a temperature of 70° F. eliminated most of the smut but did not control it completely. Treatment for the same length of time at 80° F. gave complete control of the smut but reduced the final yield by about 22 per cent.

In a fourth experiment the water soak treatment was applied at different temperatures for different periods in closed flasks and in open vessels. Montcalm barley was used in this experiment. Fifteen 8-foot rows were used for each treatment. The results obtained in this experiment are shown in Table 4.

TABLE 4.—THE INFLUENCE OF TEMPERATURE, TIME AND EXPOSURE TO AIR ON THE EFFECTIVENESS OF THE WATER SOAK TREATMENT

Temperature	Hours immersed	Treated in open dishes		Treated in closed flasks		Untreated	
		Total heads	Percentage smutted	Total heads	Percentage smutted	Total heads	Percentage smutted
° F.							
66	78	2077	0.2	2074	0		
66	95	2390	0.0	1513	0	2598	17.9
66	112	1977	0.0	878	0		
76	50	1950	0.26	1694	0		
76	60	1518	0.00	1442	0	2465	16.8
76	70	1716	0.00	873	0		
86	30	2256	0.04	2182	0		
86	40	2274	0.04	1168	0	2228	16.2
86	50	2426	0.00	261	0		

In this experiment most of the periods of treatment were longer than necessary at the temperatures chosen. A trace of smut survived after the shortest treatment in open dishes at each temperature. When the treatment was conducted in closed flasks for the same periods all of the smut was eliminated. Treatment in open dishes for the intermediate periods of time eliminated all the smut when applied at 66° F. and at 76° F., but at 86° F. a slight trace of smut survived the treatment. In every instance treatment in the closed flask reduced the number of healthy heads more than did the corresponding treatment in the open dish. The longest treatments in closed flasks caused marked reductions in yield, but on the average the corresponding treatments in open dishes reduced yields only slightly.

DISCUSSION

The Spergon treatment and the water soak treatment for loose smut (*Ustilago nuda*) of barley entail soaking the infected seed for relatively long periods of time. If these treatments are applied at temperatures that are too low, in relation to the period of immersion that is being used, the smut will be only partially eliminated. If they are applied at temperatures higher than necessary to secure control of the disease, the treated seed is likely to germinate poorly. Therefore it is important to know the minimum temperature at which these treatments should be applied, for any given length of time, to secure control of the disease.

The results of the experiments described in this paper show that there is a direct relationship between the temperature and the time required to secure control of loose smut of barley by either the Spergon or the water soak treatment. The higher the temperature, within the limits investigated, the shorter was the time necessary to obtain the desired results.

There are now three different treatments available for the control of loose smut of barley. These are the hot water, Spergon, and water soak treatments. They all cause a certain amount of injury to the seed but different lots of seed are not all affected to the same degree. The amount of injury, that any sample shows as a result of treatment, appears to be affected by a number of different factors such as the conditions under which the seed matured, physical damage to hulls and seed-coats in threshing, and the variety. This variation in response to treatment, exhibited by different lots of seed, makes it difficult to formulate an exact schedule for any of the treatments. Nevertheless, it is desirable to select schedules of treatment that will usually give complete control of the smut with as little interference with germination as is feasible.

The results presented in Table 4, as well as previous experience (4) with the water soak treatment, show that a somewhat shorter period of immersion will give control of loose smut if the seed is treated in closed containers, than is the case if it is treated in open containers. However, it seems likely that farmers will find it more convenient to apply the water soak treatment and the Spergon treatment in open tubs or tanks. Therefore the schedules of treatment suggested in the abstract of this paper refer to their use in open containers.

The reduction in the percentage of seeds that germinate following these treatments is not of as much practical importance as might appear at first

sight. Treated seed may be sown at a higher rate per acre to offset the reduction in germination. Even if it is sown at the same rate as untreated seed, if the land is free of weeds and conditions for growth are good throughout the season, the thinner initial stand may tiller sufficiently to produce as high a yield as would be produced from untreated seed.

The Spergon treatment or the water soak treatment could be applied on a large scale at community treating centres. To do this it would be necessary to have suitable tanks placed in a large room that was heated by some thermostatically controlled heating system, or as an alternative, each individual tank might be equipped with a controlled heating system. In addition a mechanical dryer would be needed. With the aid of the embryo test, seed lots that were relatively free from loose smut could be selected to supplement the treated seed and whole communities could rid themselves to a large extent of this troublesome disease and supply surrounding areas with relatively smut-free seed.

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CROWN ROT OF FRUIT TREES IN BRITISH COLUMBIA

III. RESISTANCE TRIALS ON APPLE SEEDLINGS OBTAINED FROM CONTROLLED CROSSES[1]

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[Received for publication February 1, 1954]

ABSTRACT

Apple seedlings obtained from pollen transfers between parents susceptible and resistant to the crown rot caused by *Phytophthora cactorum*, were inoculated with a mixed culture of five isolates. The disease rating (percentage of crown rot) of each group of seedlings was as follows: resistant × resistant, 7.2; resistant × susceptible, 23.5; susceptible × susceptible, 93.3. The results indicate that the factors contributing to resistance may be either partially or completely dominant, and that the character of resistance may be inherited from either the male or female parent.

INTRODUCTION

Marked differences in the resistance of apple varieties to crown rot caused by *Phytophthora cactorum* (L. & C.) Schroet. have been demonstrated (1, 2). The varieties Antonovka and McIntosh have displayed consistently high resistance, while Anis, Canada Baldwin, Winter St. Lawrence, and Yellow Transparent have been susceptible. The resistance of their open-pollinated progeny has been variable. Each group of Antonovka seedlings tested previously has been resistant but the susceptibility of groups of seedlings of the other varieties named has varied widely from that of their seed parent. However, the resistance of open-pollinated progeny from resistant varieties generally has been greater than that of open-pollinated progeny from susceptible varieties.

To determine the extent to which the resistance of each parent influences that of the progeny, controlled crosses were made between resistant and susceptible varieties and the seedlings obtained were subjected to the standard inoculation.

MATERIAL AND METHODS

The resistant varieties selected were Antonovka and McIntosh; the susceptible varieties were Winter St. Lawrence and Yellow Transparent. Blossoms were emasculated before opening, the selected pollen applied, and the blossoms caged. Seedlings obtained from these crosses were grown first in nursery rows, then transplanted, and inoculated when 5 to 6 years old.

The inoculum was a mixed culture of 4 isolates of *P. cactorum* from apple and 1 from sweet cherry. Inoculations were made below ground level by folding back a strip of bark 3/16 inches wide and about 3 inches long, smearing the inoculum on the cambium, and replacing the flap of bark. Inoculations were made in June and the extent of the infection determined

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in September. Trees that survived the first inoculation were re-inoculated the following year. The larger of the two readings obtained was used in calculating disease ratings.

Disease ratings were calculated by the method previously described (1) by which the total amount of girdling that occurred in a particular cross was expressed as a percentage of the total amount possible if all the trees of that cross had been completely girdled.

RESULTS AND DISCUSSION

Disease ratings of the seedlings obtained from these crosses are given in Table 1.

TABLE 1.—SUSCEPTIBILITY OF APPLE SEEDLINGS OBTAINED FROM CONTROLLED CROSSES, WHEN INOCULATED FOR TWO SUCCESSIVE SEASONS WITH A MIXED CULTURE OF FIVE ISOLATES OF *Phytophthora cactorum*

Cross	Number of trees	Number and amount rotted*						Disease rating	
		0	0.5	1	2	3	4		
Antonovka × McIntosh	42	14	27	1	—	—	—	8.6	
McIntosh × Antonovka	20	13	7	—	—	—	—	4.4	
Antonovka × Antonovka	16	8	7	1	—	—	—	7.0	R × R 7.2
McIntosh × McIntosh	3	1	2	—	—	—	—	8.3	
Antonovka × Yellow Transparent	54	14	31	2	1	2	4	19.2	
Yellow Transparent × Antonovka	47	24	13	3	1	—	6	18.9	
Antonovka × Winter St. Lawrence	59	26	21	—	1	2	9	23.1	R × S 23.5
McIntosh × Yellow Transparent	41	10	17	—	4	1	9	33.8	
Yellow Transparent × McIntosh	6	3	1	—	—	1	1	31.2	
Yellow Transparent × Winter St. Lawrence	28	1	1	—	—	—	26	93.3	

* 0.5—less than ¼ girdled.
1 —¼ girdled.
2 —½ girdled.
3 —¾ girdled.
4 —completely girdled.

The progeny of two resistant parents was highly resistant. None of these trees was more than one-quarter girdled, and even this amount of infection was rare. Seedlings from a cross between a resistant and a susceptible parent were moderately susceptible, and those from two susceptible parents were very susceptible. All but 2 of the 28 trees in this last group were completely girdled.

Chi-square calculations of the data show significant differences in resistance at the 1 per cent level between the groups resistant × resistant, resistant × susceptible, and susceptible × susceptible. There was no

significant difference in resistance at the 5 per cent level between each of the following crosses and its reciprocal: McIntosh × Antonovka, McIntosh × Yellow Transparent, Antonovka × Yellow Transparent. This indicates that the male parent is equally as effective as the female parent in contributing to the crown rot resistance of the progeny.

Little is known of the nature of crown rot resistance but the results of this study suggest the possibility that the factors contributing to it are either partially or completely dominant. If the factors are only partially dominant, it will be necessary, in producing seedlings resistant to crown rot, to control the pollen parent and select it with the same care as that given to the selection of the seed parent.

If the factors are completely dominant and trees can be produced that are homozygous for resistance, the resistance of their open-pollinated progeny will be assured and the production of apple seedlings resistant to crown rot for use as rootstocks will be greatly simplified.

ACKNOWLEDGMENTS

The authors wish to thank R. E. Fitzpatrick, who suggested this study, for his keen interest throughout its progress.

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NOTE ON RELATIVE CAPACITIES OF SOLUTIONS OBTAINED FROM FOREST VEGETATION FOR MOBILIZATION OF IRON

In a recent communication from this laboratory (4) it was shown that natural rainfall leachates from decomposing leaves had the capacity to mobilize iron and aluminium. Aqueous extracts obtained in the laboratory from the leaves and from the bark of trees also have been shown to possess this property (1). During the past year we have found that the solutions formed when rainfall penetrates the living forest canopy also show this characteristic.

The purpose of this note is to present comparative data on the iron-mobilization capacities of laboratory-prepared leaf extracts, of natural rainfall leachates from decomposing leaves and from A horizons of podzol soils, and of solutions dripping from the forest canopy during rainfall.

Leaf extracts were prepared by shaking 10 gm. of leaf powder obtained from air-dried leaves of the autumnal leaf-fall with 100 ml. distilled water for one hour, filtering and washing with water to a volume of 140 ml. Leachates were collected in the field as described previously (3, 4). Canopy drip solutions were obtained using the same type of equipment as for the leachates. The plant species studied were maple (Acer saccharum Marsh.) growing on a brown forest soil, beech (Fagus grandifolia Ehrh.), poplar (Populus grandidentata) and pine (Pinus strobus), the beech growing on a well-drained podzol and the poplar and pine on an imperfectly drained podzol. The A horizon materials consisted of rectangular blocks, including the A_{00} and A_0 layers and the A_2 horizon, collected under beech and under pine, cut to the dimensions of the leaching trays and fitted as snugly as possible into the latter. All the leachate and canopy drip solutions here reported upon were collected on May 22, 1954. The leaf extracts were prepared in the laboratory on the same date.

Assessment of iron-mobilizing power was made by placing 50 ml. of solution (extract, leachate or canopy drip), plus freshly-prepared, cold-precipitated ferric hydroxide gel equivalent to 66.6 mgm. of iron (Fe), in 500 ml. screw-capped bottles, adjusting the pH to approximately 5 by addition of dilute nitric acid or saturated calcium hydroxide solution, adding 10 drops of chloroform to inhibit biological action, and shaking in a horizontal position on a reciprocating shaker for 3 days, the bottles being rotated three times daily. On removal from the shaker the suspensions were centrifuged at 2000 r.p.m. for 10 minutes and aliquots of the supernatant taken for analysis. Ferric iron, and total iron after digestion with nitric and perchloric acids, were estimated in aliquots from the supernatant liquid using the thiocyanate method of Houlihan and Farina (2). The organic matter contents of the original solutions were estimated, after evaporation to dryness on the water-bath, by dichromate oxidation (5).

The data obtained for iron mobilized, that is, iron remaining in solution or suspension after shaking and centrifugation, are reported in Table 1.

TALBE 1.—IRON-MOBILIZATION CAPACITIES OF EXTRACTS, LEACHATES AND CANOPY DRIP-SOLUTIONS, EXPRESSED AS MILLIGRAMS IRON (Fe) PER GRAM OF ORGANIC MATTER

Nature of Solution	Organic Matter mgm./50 ml.	Iron Mobilized	
		Total Iron	Ferric Iron
Maple extract	309	17	13
leachate	24	19	18
canopy drip	7	43	43
Poplar extract	361	14	12
leachate	8	48	47
Beech extract	204	13	10
A-horizon leachate	2	100	100
canopy drip	7	22	22
Pine extract	368	2	2
leachate	3	13	12
A-horizon leachate	5	11	11

We conclude, on the basis of such data as are shown in Table 1, and from consideration of the undoubted influences of the microflora and the fauna of the forest floor on the nature of solutions penetrating into the soil, that conclusions respecting the mobilization of iron in soil genesis under virgin conditions will require collection for study of solutions obtained beneath the forest canopy and from materials decomposing under as natural conditions as possible.

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July 15, 1954

NOTE ON THE NAMING OF "KENYA FARMER" WHEAT

Wheat No. 338.AC.2.E.2 is a selection from Cross 338 (192.Q.2.A.L × Australian 27/2.2.1.5) made at the Plant Breeding Station, Njoro, in 1941. The 192 parent is an old Njoro hybrid developed from the cross Button's × 73.D.2.I.1.(L) made in 1933, but the ancestry and origin of both these grandparents is now lost. The Australian parent was received from R. L. Waterhouse of the University of Sydney in 1940; it is itself from the cross (Bobin × Gaza) × Bobin.

Several selections from Cross 338 were developed and tested, but were never released in Kenya because they showed no advantages over varieties already being grown at that time and were, in addition, not fully rust resistant. One selection, 338.AA.1.A.2, was, however, grown to a limited extent in the neighbouring territory of Tanganyika but it has now virtually disappeared from cultivation.

Kenya-bred wheats have been sent to many other wheat-growing countries on an exchange and co-operative basis, and it is pleasing to record that many of them have been found resistant to physiologic races of stem rust, *Puccinia graminis tritici*, prevalent in other countries. In particular, some varieties proved resistant to the rust race 15B which appeared in the United States and Canada in 1950 and which rapidly assumed epidemic proportions. Most, if not all, of the varieties in common cultivation in the Americas at that time proved highly susceptible to the new rust race; and Kenya wheats accordingly have been largely used in breeding programs designed to transfer this resistance to local varieties.

In a recent personal communication, R. F. Peterson, Officer-in-Charge of the Cereal Breeding Laboratory at Winnipeg, Manitoba, informed the author that the Kenya wheat 338.AC.2.E.2 is being used extensively in Canada as a source of rust resistance in breeding work in that country. The variety is considered the most important source of stem rust resistance at present available to Canadian workers; and there is as yet no race of stem rust known to damage the variety to any appreciable extent in North America. It is also useful for its resistance to leaf rust, *P. triticina*. In view of its importance and widespread use both in Canada and in the United States, a request has been put forward by Dr. Peterson that it would be useful to name the variety.

Many of the wheats in Kenya have not so far been named since experience has shown that each has had a comparatively short life, due to susceptibility to new physiologic stem rust races. However, a name has advantages over a complicated number as being less liable to mutilation and is also, as a rule, more pleasant.

It has been agreed, therefore, to name this variety; and in conformity with custom the first name "Kenya" is to be retained as in the case of varieties already named, such as: Kenya Governor, Kenya Standard, Kenya Settler, and Kenya Ploughman.

The name given to 338.AC.2.E.2 is "Kenya Farmer".

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July 30, 1954.